SOME APPLICATIONS OF GENETICS IN DENTISTRY

J.A. SOFAER

DSc

UNIVERSITY OF EDINBURGH

1983

ABSTRACT

Genetics is fundamental to an understanding of differences between individuals and between populations. Common minor differences are found within what is generally accepted as the normal range of variation, while relatively unusual but more major differences may be appropriately considered under the heading of pathology. Both major and minor differences occur in terms of structure, function or susceptibility to disease. This submission contains examples of such differences and their exploitation or analysis, most of which fall within the general field of dentistry.

A number of observations of inherited dental abnormalities in man and experimental animals are included. These indicate that there can be compensatory interaction between neighbouring tooth germs during development. Based on this interaction, a model to account for differential evolutionary reduction of tooth size is proposed. Studies of regional differentiation in the mouse vertebral column are described, the vertebral column being a series of homologous structures divided into morphological classes in the same way as heterodont dentitions. The effects of a number of inherited disorders of the axial skeleton indicate that vertebral class boundaries in the mouse are established at a very early stage, even before somite formation. The use of dental morphology for population discrimination is discussed in relation to studies of the genetics of dental morphological variation, and a population comparison in which the discriminating power of dental morphology was tested against that provided by known genetic variants.

Studies of inherited iron-deficiency anaemia in the mouse are described. They show that the disorder is associated with thinner lingual epithelium than normal and possibly with increased susceptibility to oral candidosis. Different strains of candida produced different levels of oral colonisation and infection in normal mice, suggesting that susceptibility to candidosis may be related to variation in the microorganism as well as the host. A human family

study of Paget's disease of bone is reported. The results are consistent with the hypothesis that Paget's disease is caused by a common virus, with genetic variation for susceptibility to the disease. Also in man, a comparison between carriers of X-linked hypohidrotic ectodermal dysplasia (in whom manifestations of the disease may be limited to minimal hypodontia) and females with hypodontia for other reasons indicates that carriers may be distinguished from among female hypodontia cases in general by means of a reduced sweat pore count.

In the past, various genetic principles have been misapplied in dentistry. Two critiques of such misapplications are included, together with contributions to a review of current dental research, undergraduate and postgraduate dental texts, and a major new medical genetics text.

DECLARATION

The work described in papers 1, 2 and 17 was supervised by Dr D.S. Falconer and presented in the form of a Ph.D. thesis at the University of Edinburgh in 1968.

Joint authorship

Paper 6 - The first author carried out the analysis of data collected by Dr J.H. Shaw.

Paper 7 - The study was originated and carried out by the first author with histopathological advice from Professor J.C. Southam.

Paper 10 - The study was originated by the first author in collaboration with Dr J.D. Niswander, using data collected by Dr D.W. Runck. Dr C.S. Chung carried out the statistical analysis.

Papers 15 & 18 - The studies were originated and carried out by the first author with statistical advice from Dr C.J. MacLean, in collaboration with and using data collected by Dr H.L. Bailit.

Paper 19 - The study was originated by the first author in collaboration with Dr J.D. Niswander. The first author collected the data and undertook the analysis with advice from Drs C.J. MacLean and P.L. Workman.

Paper 20 - The study was originated by Professor J.C. Southam. The second author and Professor Southam supervised the collection and analysis of data by Mr B. Steele.

Paper 21 - The study was originated collaboratively. The first author was responsible for the mouse stocks and made a substantial contribution to the analysis. Dr W.P. Holbrook was responsible for the microbiological and Professor J.C. Southam for the histopathological aspects of the work.

Paper 22 - The study was originated by Dr W.P. Holbrook. The second author was responsible for the mouse stocks and made a contribution to the analysis.

Papers 23 & 28 - The studies were originated by Professor A.E.H. Emery. The first author made a substantial contribution to the collection and analysis of the data.

Paper 26 - The study was originated by the first author. Statistical analysis was by Dr C.J. MacLean.

In all cases of joint authorship, the first author took the major initiative in writing the paper.

Single authorship

All other material submitted is the work of the candidate alone, with the exception of minor assistance indicated by acknowledgements in the individual papers as appropriate.

CONTENTS

THE CONTROL OF DENTAL DEVELOPMENT

SINGLE GENE EFFECTS

Analysis of the effects of single mutant genes can give insight into the control of normal development. For example, studies of the mouse mutants 'tabby', 'crinkled', 'downless' and 'sleek' (the latter two of which have recently been shown to be allelic), the mouse mutant 'crooked' and a strain of rice rat, provide evidence for interaction between tooth germs during development and point to factors that may contribute to the initiation of tooth germs. Dental abnormalities in the hypophosphataemic mouse appear to be equivalent to those in the corresponding human condition, suggesting that this mutant may be a useful model for studying the origin and treatment of the dental manifestations of this disease.

J. Embryol. exp. Morph. Vol. 22, 2, pp. 181–205, September 1969
Printed in Great Britain

Aspects of the tabby–crinkled–downless syndrome

I. The development of tabby teeth

By J. A. SOFAER[1]

The Institute of Animal Genetics, West Mains Road, Edinburgh

The sex-linked gene tabby, *Ta* (Falconer, 1953), and two autosomal mimics of tabby, crinkled (*cr*, linkage group XIV) (Falconer, Fraser & King, 1951; King, 1956) and downless (*dl*, linkage group IV) (*Mouse News Letter*, 1960, 1966) each produce a similar mutant syndrome involving the coat and dentition of the mouse. Studies on the coats of tabby and crinkled mice point to a timed gene effect causing suppression of formation of new hair follicles between 12½ and 17 days of gestation and again from birth onwards, with a reduction in the rate of growth of the follicles that do form (Falconer *et al.* 1951). Associated with this is a reduction in hair calibre and a lack of differentiation of the coat into hair types (Grüneberg, 1966*b*). A model to explain the timed action of the tabby gene has been proposed by Dun (1959).

The teeth of tabby and crinkled mice have been described in detail by Grüneberg (1965, 1966*a*), and a comparative study of the effects of two alleles of tabby, *Ta* and Ta^c, crinkled and downless, has been made by Sofaer (1969). In all mutant homozygotes and tabby hemizygotes incisors may be reduced or absent. The first and second molars are generally reduced and their morphology is characteristic. Third molars are often absent. The dentitions of heterozygotes for each of the genes may contain normal teeth, frankly mutant teeth, and teeth combining characteristics of both the normal and mutant phenotypes. All three types of tooth may be present in the same animal. A further feature of the heterozygote dentition is the rare occurrence of an additional molar tooth. Grüneberg (1966*a*) has called this phenomenon 'twinning' and has described three categories:

(i) *Overt twinning*, where there are four molars in a row instead of the usual three. The normal first molar is represented by two twin teeth, the anterior of which tends to be the smaller.

(ii) *Concealed twinning*, which is similar to overt twinning except that the third molar is absent. There are therefore, only three teeth in the row, as in the normal mouse, but the first two can usually be diagnosed as twins with reasonable certainty on the basis of the appearance of twins in the overt cases.

(iii) *Incomplete twinning*, which is recognized by the presence of additional

[1] *Author's address:* National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

cusps and roots, and by anteroposterior elongation and pinching in of the sides of the first molar crown. In one case described the twins had separate crowns, but there was a single root that was common to both.

Grüneberg (1966*a*) suspected that twinning may also take place in homozygotes and tabby hemizygotes. Examples of twinning in homozygotes and hemizygotes, including incisor twinning, have been found both in the embryological material which will be described presently, and amongst the adult dentitions examined by Sofaer (1969).

The present investigation is concerned with the development of tabby (*Ta*) teeth only, with particular reference to the phenomenon of twinning. There is no reason to suppose that the development of the teeth of Ta^c, crinkled, or downless mice is fundamentally any different, so conclusions drawn here could be applied with equal confidence to all the genes. An attempt has been made to explain dental aspects of the syndrome in the light of what is known of the development of the coat so that both tooth and hair defects can be considered in terms of the principle of 'unity of gene action' (Grüneberg, 1943*a*).

MATERIALS AND METHODS

The *A* strain background has been found to favour the expression of incisor abnormality in tabby hemizygotes (Grüneberg, 1965), as well as the expression of molar abnormalities in heterozygotes (Sofaer, 1969). Material for sectioning was accordingly obtained as follows. *A* strain males mated to *A* strain females provided a control group of litters. *A* strain males mated to homozygous tabby females provided litters of mixed heterozygotes and tabby hemizygotes. The majority of homozygous tabby mothers were from stock, but a few were the result of one or two crosses to the *A* strain. It was originally intended to use these latter animals exclusively, but poor fertility made this impossible. The majority of litters examined were therefore heterozygous for the *A* strain background, but a few were nearly homozygous. There were no obvious differences between these two types of litter.

Animals were caged one male to a maximum of three females. No suckling females were used. Matings were examined for births and females were examined for vaginal plugs between 9 and 10 a.m. Material was collected between 10 a.m. and midday. The day on which a plug was found was regarded as day zero. Litters were collected at 2-day intervals from day 13 to day 29. Eight post-partum litters were used for which plug dates were not known. Birth was then taken as the criterion of age and was taken to have occurred at 20 days. (Of the 25 post-partum litters collected for which plug dates were known, one was born on day 18, twelve on day 19, eleven on day 20, and one on day 21). The ages of litters collected before birth were checked by examination of the external features of the embryos (Grüneberg, 1943*b*). All animals of the *A* strain litters, but heterozygotes only of the mixed litters, were checked in this way.

Tabby hemizygotes and heterozygotes of 13–day litters were separated on the basis of presence or absence of the postorbital tubercles. These are the first signs of the developing postorbital vibrissae which are very nearly always absent in hemizygotes and present in heterozygotes. There was no difficulty in separating the two types at this stage. For classification of older individuals additional criteria were adopted: the degree of eruption of body hairs; the number of supraorbital vibrissae; and in post-partum litters, the sex of the individual. Although a postorbital fibre is rarely present in tabby hemizygotes at birth, Dun (1959) found that at 5 days after birth there is invariably a small, slow growing, atypical fibre at this site. Such fibres are lost in the hair of the fully grown coat. Fibres of this type were found in the present material but were easily distinguishable from those of heterozygotes. The additional use of the other criteria at this stage made the possibility of misclassification very remote.

Table 1. *The numbers of animals of each genotype sectioned at each stage, followed in parentheses by the numbers of litters from which they were taken*

	Genotype		
Stage	*A* strain	*Ta+*	*Ta*
13 days	5 (3)	5 (2)	5 (2)
15 days	5 (3)	5 (4)	5 (3)
17 days	5 (3)	9 (3)	5 (3)
19 days	5 (2)	7 (3)	5 (3)
21 days	5 (3)	9 (3)	5 (3)
23 days	2 (2)	5 (3)	4 (2)
25 days	2 (1)	2 (1)	5 (3)
27 days	3 (1)	5 (3)	4 (2)
29 days	0	5 (2)	5 (2)

All individuals were classified prior to fixation after examination under a dissecting microscope. The 13- and 15-day embryos were fixed whole. Seventeen-day embryos were decapitated and the heads only were fixed. The classification of these embryos was checked again after fixation and prior to further processing. Individuals of 19 days and older were decapitated and the heads were skinned before fixation. Classification of these animals could therefore not be checked subsequently. There were very few cases where classification was in doubt. These were mainly instances where a postorbital fibre was present on one side but not on the other. Animals of this type were rejected. Examination of the prepared material, in the light of what is known to occur in adult animals, provided no evidence to suggest that any misclassification had been made.

All litters were fixed in Bouin's fluid. Litters of 19 days and older were decalcified in 5 % nitric acid. All material was embedded in paraffin wax, serially sectioned at 10 μ in the sagittal plane, and stained with haematoxylin and eosin.

A total of 127 animals from sixty-five litters were prepared and examined. The numbers of each genotype sectioned at each stage are shown in Table 1.

RESULTS

These will be considered in four sections: incisors, lower first and second molars, upper first and second molars, and third molars. The findings in the control group were comparable with those of previous workers (Gaunt, 1955, 1956, 1961; Cohn, 1957; Hinrichsen, 1959; Hay, 1961).

1. *Incisors*

Heterozygotes showed no differences from the controls and are therefore not considered here.

At 13 days there was no definite difference between the tooth rudiments of *Ta* and control animals except that, on average, the *Ta* rudiments were probably a little smaller. At 15 days a definite difference was apparent. The *Ta* tooth germs were obviously smaller than the controls and were barely invaginated. (Fig. 1, compare A and B).

EXPLANATION OF FIGURES

LL	labial lamina	*D*	dentine
DL	dental lamina	*PA*	pre-ameloblasts
IE	internal enamel epithelium	*E*	enamel
EE	external enamel epithelium	*S*	supernumerary tooth germ
O	odontoblasts		

Unless otherwise stated the left of each illustration is anterior and the right is posterior.

FIGURE 1.

A. Control lower incisor at 15 days. The tooth germ is in the early bell stage with early differentiation of the internal and external enamel epithelia.

B. Tabby hemizygote lower incisor at 15 days. The downgrowth of labial lamina is comparable with the control, but almost no invagination of the tooth germ has taken place.

C. Control lower incisor at 19 days. Dentine formation has started and the pre-ameloblasts are well differentiated.

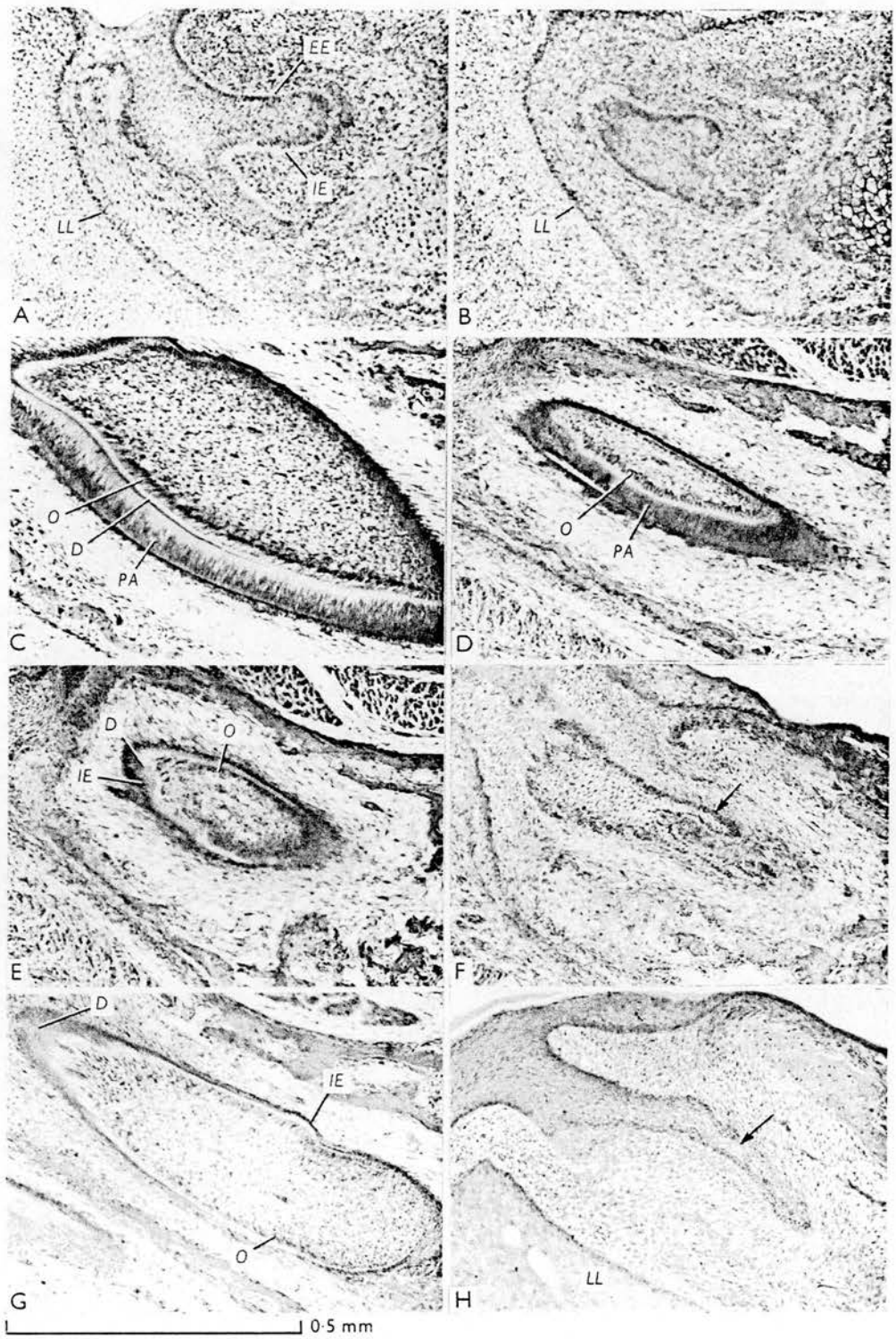
D. Tabby hemizygote lower incisor at 19 days. An example of a more or less well differentiated tooth germ of abnormal size and shape.

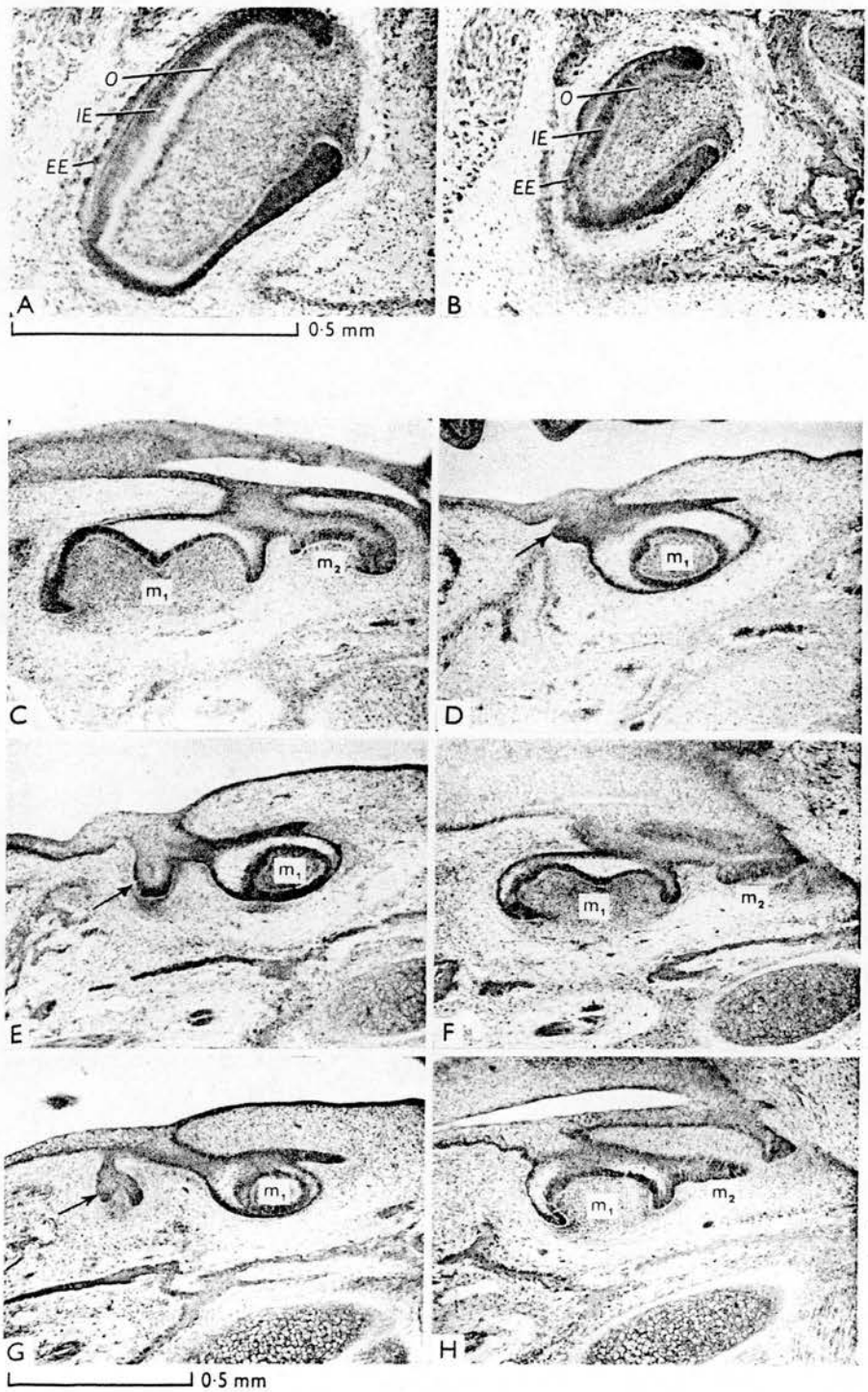
E. Tabby hemizygote lower incisor at 19 days. A poorly differentiated example with degenerating internal enamel epithelium and abnormal odontoblasts.

F. Tabby hemizygote lower incisor region at 19 days, showing an undifferentiated lower incisor rudiment (indicated by the arrow).

G. Tabby hemizygote lower incisor at 27 days. A poorly differentiated example which has grown and maintained its structure.

H. Tabby hemizygote lower incisor region at 27 days, showing retained remnants of degenerating dental epithelium (indicated by the arrow).


EE
IE
LL
A
LL
B
O
D
PA
C
O
PA
D
D
O
IE
E
F
D
IE
O
G
LL
H
0·5 mm


O
IE
EE
A
B
0·5 mm
m1
m2
C
D
E
F
G
H
0·5 mm

There was a striking difference in intensity of abnormality between upper and lower jaws. This is consistent with what has been found in fully formed dentitions. All the upper incisor germs looked as if they would have formed teeth. By contrast there was a wide range of expression in the lowers varying from near normality to degeneration. From 19 days three distinct categories of abnormal lower incisor germ were discernible:

(i) More or less well differentiated though variable in size and shape (Fig. 1 D, compare with control C).

(ii) Poorly differentiated (Fig. 1 E), in which the internal enamel epithelium showed signs of degeneration and where the odontoblasts were abnormal. No enamel but some dentine was formed. Such partially differentiated germs increased in size up to the latest stage examined (Fig. 1 G).

(iii) Undifferentiated (Fig. 1 F), in which the dental epithelium showed no sign of morphodifferentiation or further histodifferentiation and appeared to be undergoing degeneration. Epithelial remnants were retained up to the latest stage examined (Fig. 1 H).

Table 2 shows the relative frequencies of these categories of abnormal lower incisor germ at different stages.

In the upper jaw the relatively small size of the *Ta* germs was maintained at all stages and was associated with delayed histodifferentiation (compare Figs. 2 A and B).

It can therefore be concluded that, in *Ta* animals, growth and histodifferentiation of developing incisor germs may be retarded; that in more severely affected cases, found only in the lower jaw, the internal enamel epithelium is the first tissue to suffer degeneration; and that in the most severely affected cases there is

---

FIGURE 2

A. Control upper incisor at 17 days.

B. Tabby hemizygote upper incisor at 17 days. The bell is smaller and histodifferentiation much less advanced than in the control.

C. Control lower first and second molar germs at 17 days.

D. Control lower first molar germ at 17 days, sectioned lingually to show the normal anterior extension of dental lamina (indicated by the arrow).

E. Tabby heterozygote at 17 days with the first molar sectioned lingually. There is a large bud of dental lamina anteriorly (indicated by the arrow).

F. The same example as in E, sectioned further buccally to show the maximum diameter of m_1 and m_2, which are smaller than in the control.

G. Tabby hemizygote at 17 days with the first molar germ sectioned lingually. There is an anterior bud of dental lamina showing some invagination (indicated by the arrow).

H. The same example as in G, sectioned further buccally to show the maximum diameter of m_1 and m_2, which are smaller than in the heterozygote shown in F, and much smaller than in the control.

Table 2. *The numbers of* Ta *lower incisor germs in three categories of abnormality observed at different stages*

Stage	Category		
	Well differentiated. Variable size and shape	Poorly differentiated	Undifferentiated
19 days	5	1	4
21 days	0	0	10
23 days	2	1	5
25 days	5	2	3
27 days	3	1	4
Total	15	5	26

a complete lack of differentiation and epithelial growth very nearly, if not completely, ceases.

2. *Lower first and second molars*

Both the heterozygote and hemizygote groups showed differences from the controls and are therefore both considered here.

At 13 days there were no detectable differences between the tooth rudiments of *Ta*, *Ta*+ and control animals. At 15 days differences became apparent. At this and subsequent stages *Ta* tooth germs were generally smaller than the controls and more bulbous in shape. Small size was sometimes associated with

FIGURE 3

A. Control lower first molar at 19 days.

B. The same example as in A, sectioned further buccally and further posteriorly to show the maximum diameter of m_2.

C. Control lower first molar at 19 days sectioned lingually to show the normal anterior extension of dental lamina at this stage (indicated by the arrow).

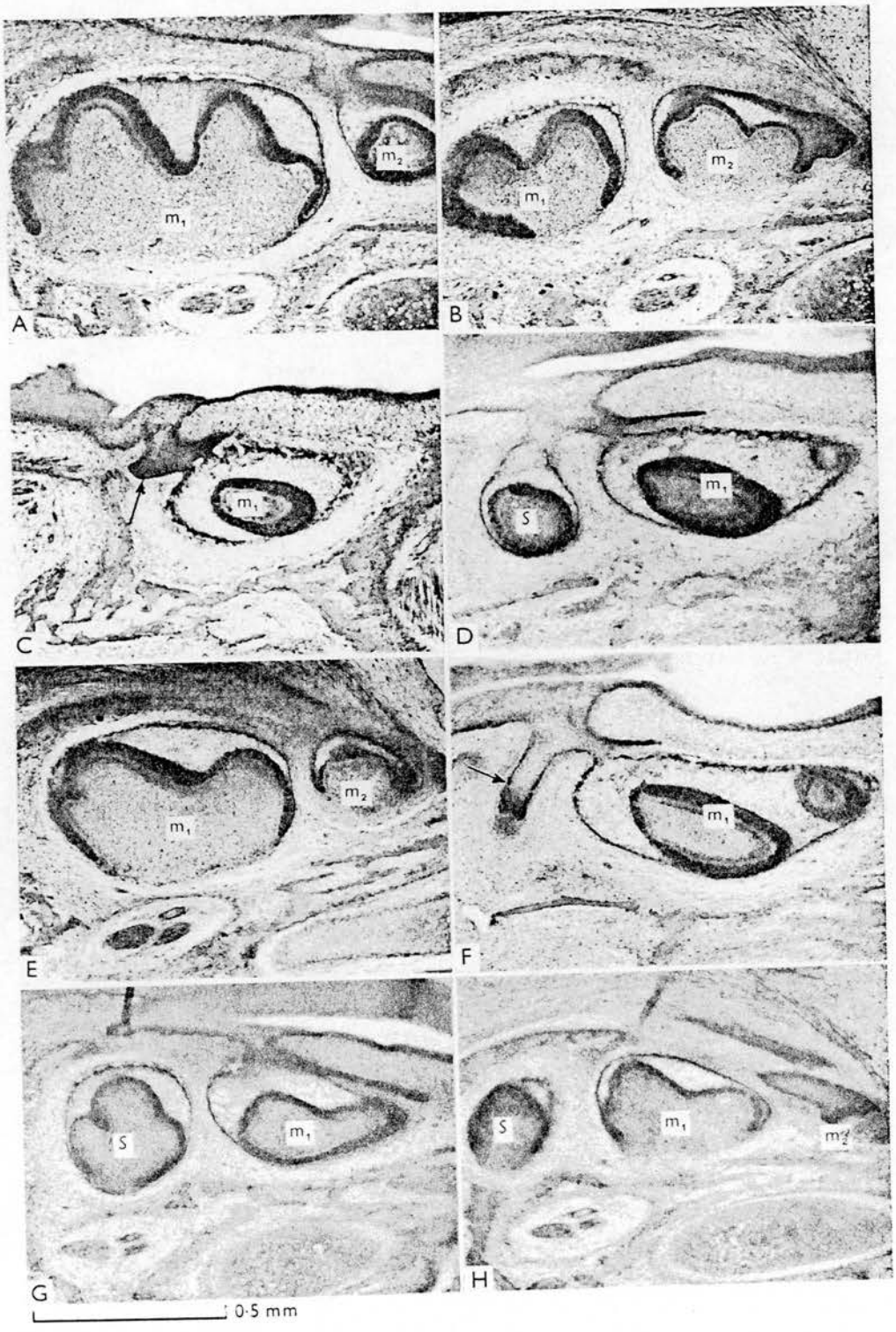
D. Tabby heterozygote at 19 days with m_1 sectioned lingually. There is a small supernumerary germ anteriorly with its own laminal connexions.

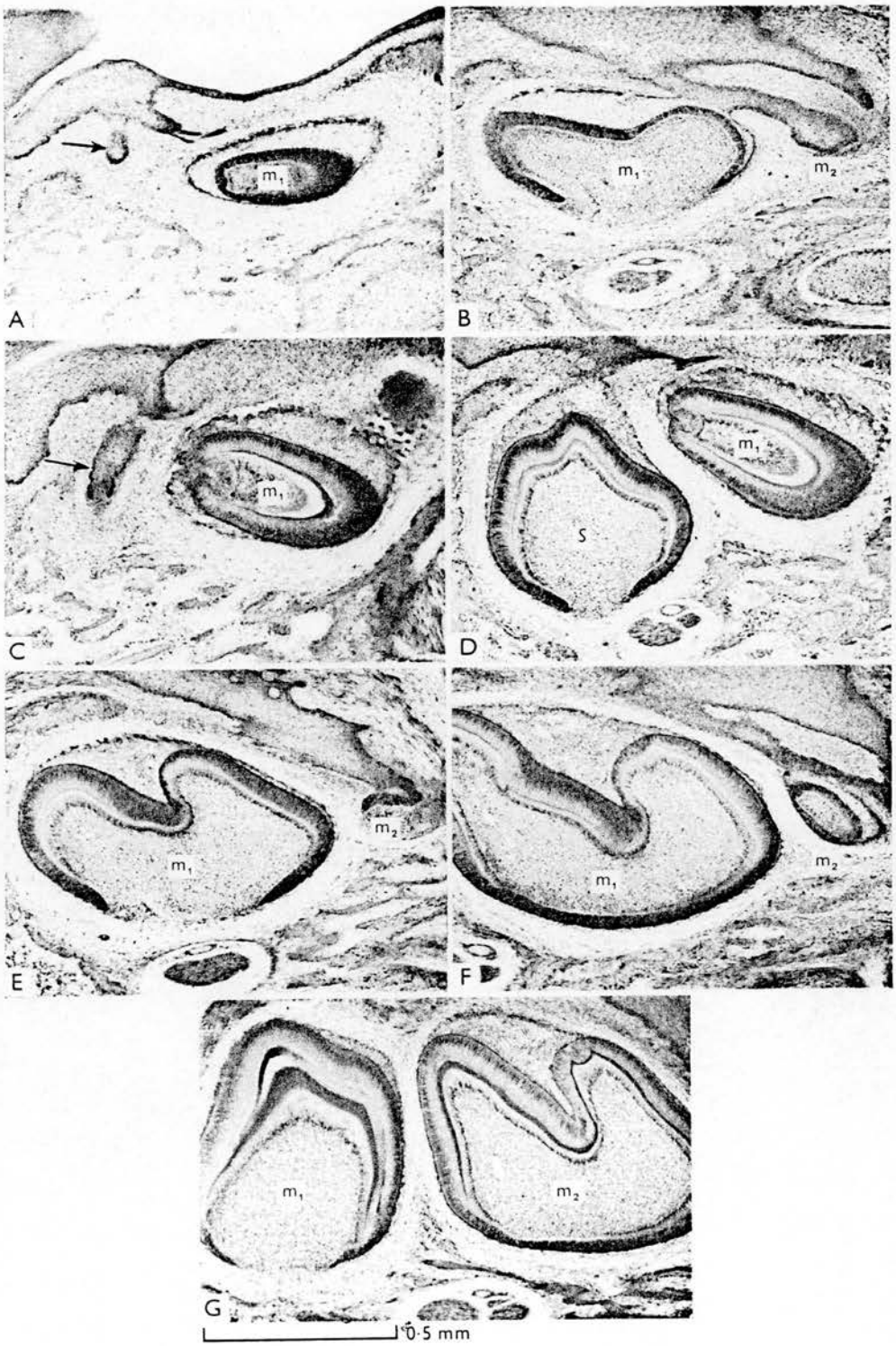
E. The same example as in D, sectioned further buccally and further posteriorly to show the maximum diameter of m_1 and m_2, which are smaller and less well differentiated than in the control.

F. The same animal as shown in D and E, but the opposite side. There is considerable epithelial downgrowth anteriorly (indicated by the arrow). Comparison with the opposite side suggests that this was an unsuccessful attempt to form a supernumerary tooth germ.

G. Tabby hemizygote at 19 days, showing the maximum diameter of an anterior supernumerary germ. The first molar is sectioned rather lingually.

H. The same example as in G, sectioned further buccally and further posteriorly to show the maximum diameter of m_1 and m_2.


A
m1
m2
B
m1
m2
C
m1
D
S
m1
E
m1
m2
F
m1
G
S
m1
H
S
m1
m2
0·5 mm


A
m1
B
m1
m2
C
m1
D
m1
S
E
m1
m2
F
m1
m2
G
m1
m2
0·5 mm

delayed histodifferentiation. Similar but less severe abnormalities were present in some heterozygotes. Examples of interaction between developing first and second molars were observed. Poor development of m_1 was sometimes associated with an enlarged m_2 in which differentiation was sometimes more advanced than in the control m_2. However, m_2 never appeared to be as advanced as m_1. At no stage was there any evidence of division of the first molar germ into two in either *Ta*+ or *Ta* animals.

A feature of the control animals was a small extension of the dental lamina anteriorly from the point of origin of the first molar germ, and somewhat lingually (Figs. 2D; 3C). In a few *Ta*+ and *Ta* animals there was proliferation of this extension of lamina to form an epithelial downgrowth anterior to the developing m_1 (Figs. 2E, G). In some of these cases a supernumerary tooth germ was formed (Fig. 3D, G) and in others the epithelial downgrowth appeared to regress (Fig. 3F; Fig. 4A, C). There was evidence of interaction between this epithelial downgrowth and the developing m_1 and m_2, whether or not a supernumerary germ was formed. The presence of a potential or developing supernumerary germ was associated with a small m_1 and a small m_2 (Fig. 2, compare F, H with control, C; Fig. 3, compare E, H with control, A, B). Cases of degenerating epithelial downgrowths showed signs of the same interaction though to a lesser extent (compare Fig. 4A, B with Fig. 3G, H, and control, A, B).

Whether the most anterior germ was a first molar or a supernumerary was decided after comparison of all the molar tooth germs on that side (e.g. Fig. 3D, E; Fig. 4D, E); of the affected side with the opposite side, which was generally

---

FIGURE 4

A. Tabby hemizygote at 19 days, showing an anterior downgrowth of dental lamina which suggests an unsuccessful attempt to form a supernumerary tooth germ.

B. The same example as in A, sectioned further buccally and further posteriorly to show the maximum diameter of m_1 and m_2.

C. Tabby heterozygote at 21 days with the first molar sectioned lingually. There is a degenerating downgrowth of dental lamina anteriorly (indicated by the arrow).

D. Tabby hemizygote at 23 days, showing the laminal connexions of an anterior supernumerary with m_1 sectioned lingually. Dentine formation in these two teeth is about equally advanced.

E. The same example as in D, sectioned further buccally and further posteriorly to show the maximum diameter of m_2, which is much smaller and less advanced than the m_2 of the opposite side (see F), and possibly would not have progressed to form a tooth.

F. The same animal as in D and E, but the opposite side showing m_1 and a small m_2. There was no sign of an attempt to form a supernumerary tooth germ anteriorly.

G. Tabby hemizygote at 25 days, showing the maximum diameter of m_1 and m_2. Enamel formation in m_1 is much further advanced than in m_2, but m_1 is much smaller than m_2.

more normal (e.g. Fig. 3D with F; Fig. 4E with F); and of the affected animal with others at the same stage (e.g. Fig. 3D, E with control, A, B).

It was considered that a supernumerary germ could never be larger or more advanced than the m_1 it preceded, although after 19 days histodifferentiation in these two teeth appeared to be about equally advanced (Fig. 3D, G; Fig. 4D). It was also considered that m_1 would always be in a more advanced state of histodifferentiation than m_2. However, m_1 and m_2 were sometimes observed to be of almost equal size, and in one case the tooth taken to be m_1 on the basis of the thickness of its enamel and dentine was considerably smaller than m_2 (Fig. 4G).

Table 3. *The total numbers of* Ta+ *or* Ta *lower first molars examined at each stage* (*N*), *the numbers of cases where there was proliferation of the anterior lamina without supernumerary tooth germ formation* (*P*), *and the numbers of cases where a supernumerary germ was found* (*S*).

	Ta+			*Ta*		
Stage	N	P	S	N	P	S
17 days	17	3	0	9	1	0
19 days	14	1	2	10	1	1
21 days	18	1	0	10	0	0
23 days	10	0	0	8	0	1
25 days	4	0	1	10	0	0
Total	63	5	3	47	2	2

Table 3 shows the total numbers of developing *Ta+* and *Ta* first molars examined at different stages, the numbers of cases where proliferation of the anterior lamina without supernumerary tooth germ formation was observed, and the numbers of cases where a supernumerary germ had become established.

FIGURE 5

A. Control upper first molar germ at 15 days, showing the maximum concavity of the bell.

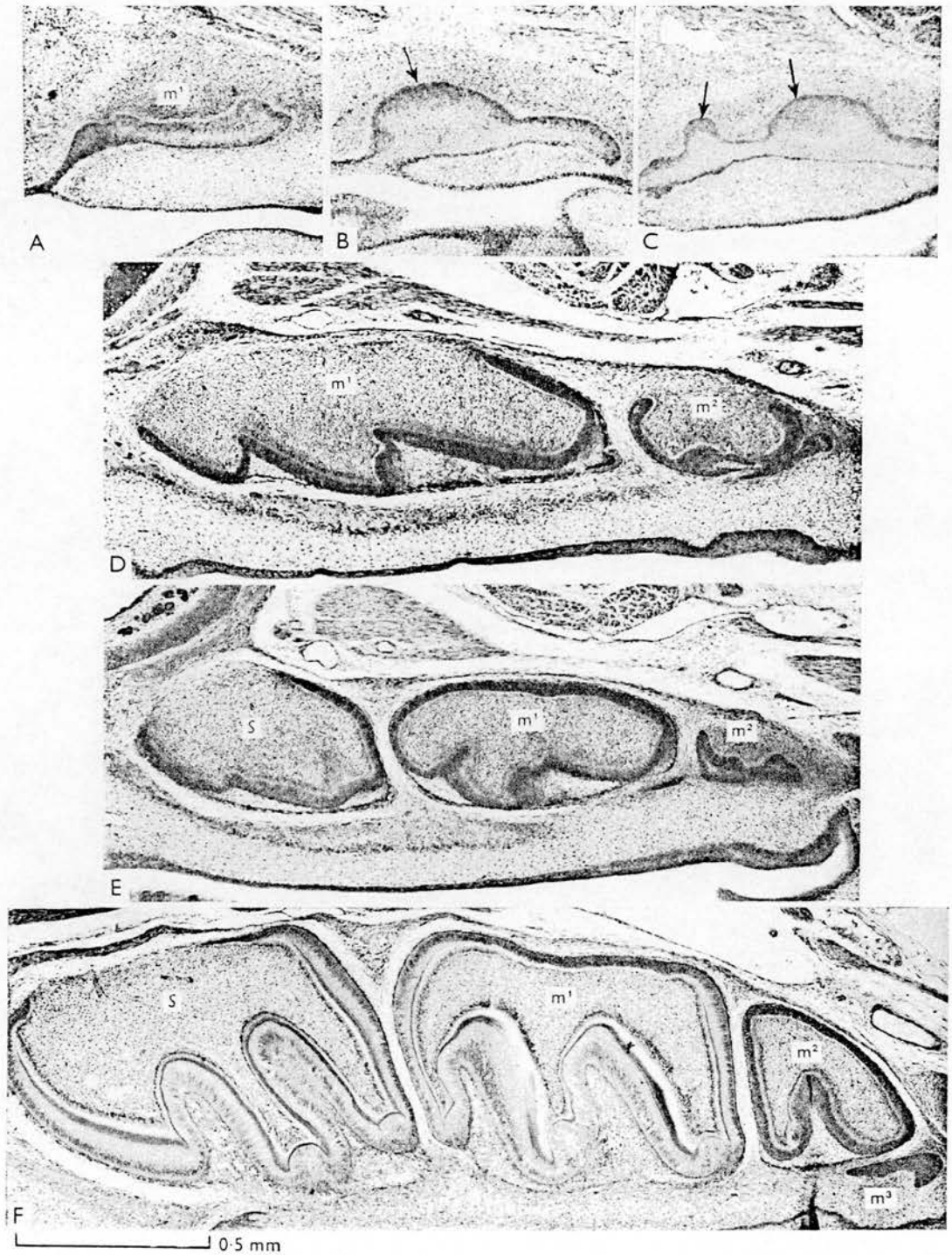
B. The same example as in A, sectioned further buccally to show the maximum height of the buccal margin of the bell (indicated by the arrow).

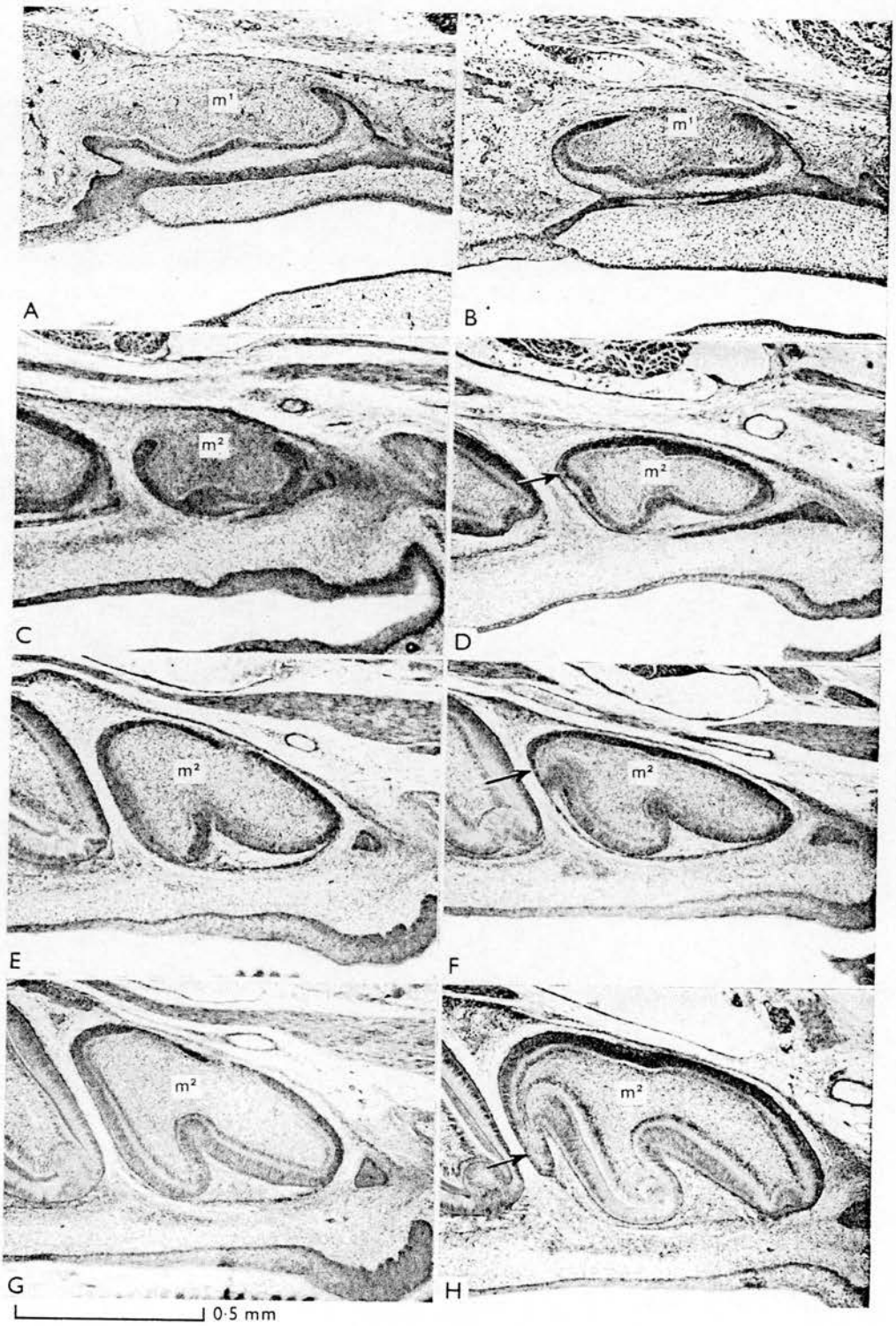
C. Tabby heterozygote at 15 days, showing the maximum heights of the buccal margins of two bells (indicated by the arrows).

D. Control upper first and second molar germs at 19 days.

E. Tabby heterozygote at 19 days, showing an anterior supernumerary with m^1 and m^2. The total anteroposterior length of these three germs is similar to that of the normal m^1 and m^2 in D.

F. Tabby heterozygote at 23 days, showing an anterior supernumerary with m^1, m^2, and the rudiment of m^3.


m¹
A
B
C
m¹
m²
D
S
m¹
m²
E
S
m¹
m²
m³
F
0·5 mm


m¹
A
m¹
B
m²
C
m²
D
m²
E
m²
F
m²
G
m²
H
0·5 mm

3. *Upper first and second molars*

Abnormalities of the upper molars were less striking than those of the lowers. Just as in the lowers, there was no evidence of division of a first molar germ into two. However, only one example of what appeared to be early supernumerary development was found (Fig. 5; compare C with control, A, B). Amongst individuals of the more advanced stages there were two examples of established supernumerary teeth (Fig. 5E, compare with control, D; Fig. 5F). These three cases were all in the *Ta*+ group. No upper supernumeraries were observed in the *Ta* group.

The 'rampart' of the tabby upper second molar (Grüneberg, 1965) has been regarded as a reaction to the small size of m^1. Figure 6A, B shows the difference in size between normal and tabby upper first molars at 17 days. The rampart starts as an anterior outgrowth which is first noticeable at 19 days (Fig. 6D; compare with control, Fig. 6C), and which subsequently becomes bent occlusally as it increases in size and as the space between m^1 and m^2 closes (Fig. 6F, H; compare with control, E, G). The attempt at compensation therefore appears to be at least partially frustrated by lack of space.

4. *Third molars*

A difference between rudiments which were presumed to be destined for regression and those which looked as if they would form teeth started to be detectable at 25 days and was definite at 27 days. The rudiments which were destined for regression did not invaginate to form bells. No cases of regression were found in the controls, though absence of lower third molars does occur in the *A* strain at a low frequency. No bell was formed by any of the *Ta* m_3 rudiments at 27 and 29 days. About half the *Ta* m^3 rudiments had formed bells at these stages. Most of the *Ta*+ m_3 and all of the *Ta*+ m^3 rudiments had formed

---

FIGURE 6

A. Control upper first molar at 17 days.

B. Tabby hemizygote upper first molar at 17 days.

C. Control upper second molar at 19 days.

D. Tabby hemizygote upper second molar at 19 days, showing the first sign of the developing rampart (indicated by the arrow).

E. Control upper second molar at 21 days.

F. Tabby hemizygote upper second molar at 21 days, showing further development of the rampart (indicated by the arrow). Histodifferentiation appears to be a little more advanced than in the control.

G. Control upper second molar at 23 days.

H. Tabby hemizygote upper second molar at 23 days, showing further development of the rampart (indicated by the arrow). Histodifferentiation is more advanced than in the control.

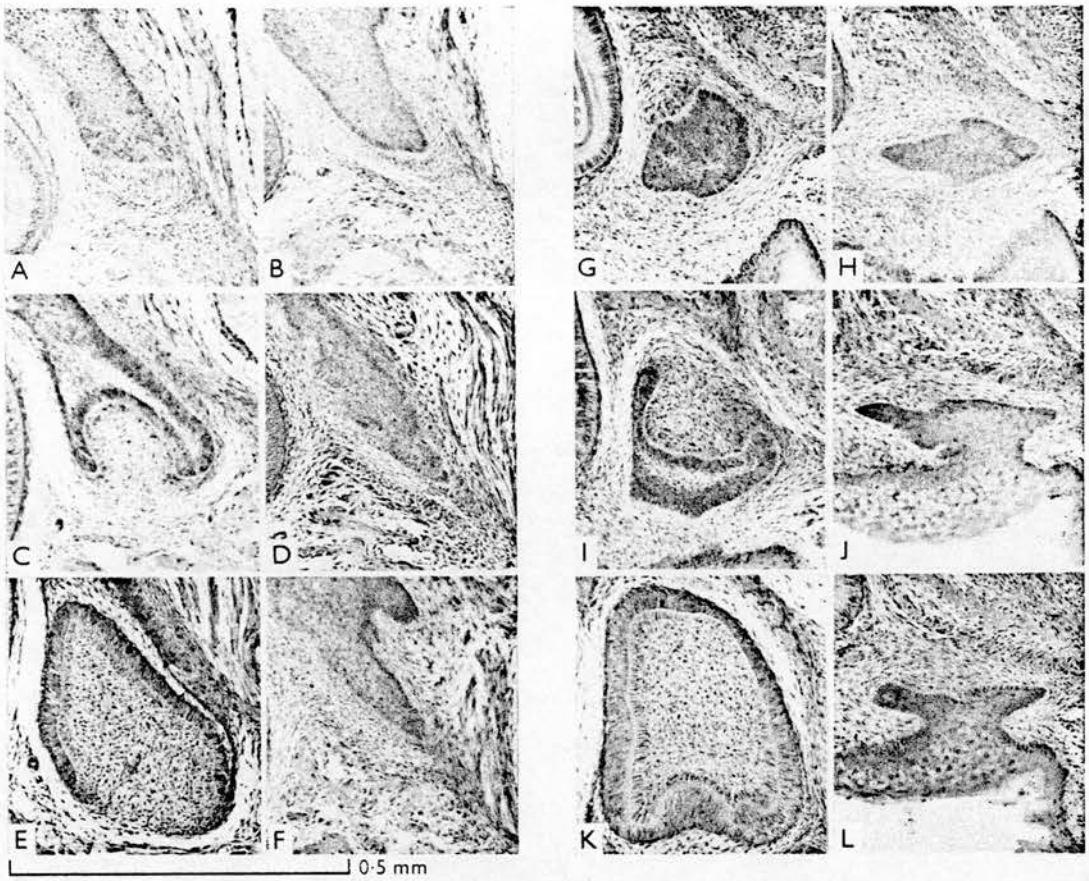


FIGURE 7

Lower third molars

A. Control rudiment at 25 days.
B. Tabby hemizygote rudiment at 25 days.
C. Control at 27 days.
D. Tabby hemizygote at 27 days.
E. Tabby heterozygote at 29 days.
F. Tabby hemizygote at 29 days.

Upper third molars

G. Control rudiment at 25 days.
H. Tabby hemizygote rudiment at 25 days.
I. Control at 27 days.
J. Tabby hemizygote at 27 days.
K. Tabby heterozygote at 29 days.
L. Tabby hemizygote at 29 days.

bells at these stages (Fig. 7). These findings are comparable with those of Grewal (1962), who demonstrated a similar embryological basis for the absence of third molars in *CBA* and crooked tail mice.

DISCUSSION

1. *General observations*

In tabby hemizygotes the general effect on the developing tooth germs appeared to be one of reduced rate of growth and delayed histodifferentiation. The effect on the lower incisors was the most severe. Sometimes no tooth at all

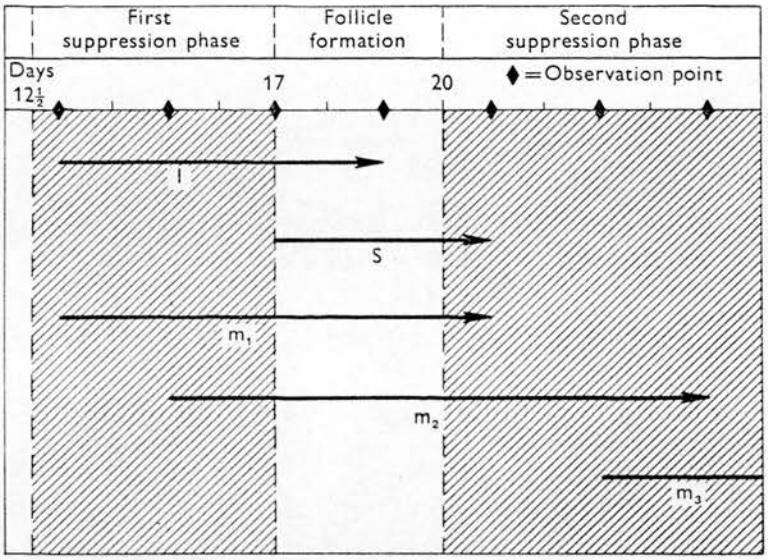


Fig. 8. The relationship of the developmental sequence of the lower teeth to the phases of hair-follicle formation and suppression. The arrows represent the time taken for the course of development of each tooth from the appearance of a definitive epithelial bud to the first appearance of calcified dentine, observations being made every 2 days from day 13. No example of a supernumerary tooth was found at the 21-day stage. The point of the S (supernumerary) arrow has been arrived at by interpolation from 19- and 23-day examples. I = incisor.

was formed, and sometimes there was an intermediate condition where some dentine but no enamel was formed. In the case of the molars there was no evidence to suggest that enamel formation is ever prevented or that a first molar is ever completely suppressed. It did seem likely that complete suppression could be the rare fate of some lower second molar germs (Fig. 4E). Regression of third molar germs was a frequent occurrence. Similar but less severe effects were observed in the molars of some heterozygotes.

'Overt twinning' in the lower jaw was found to be produced by the *de novo* development of a supernumerary tooth from an overgrowth of a normal

anterior extension of dental lamina. Direct evidence for this in the upper jaw was limited, though observations here were not inconsistent with the lower jaw findings. There was no evidence of division into two of any first molar germ, either upper or lower, at any stage. As the failure of third molar rudiments to form bells was observed many times it is reasonable to assume that 'concealed twinning' does occur. Examples of developing supernumerary teeth were found in both upper and lower jaws of heterozygotes, but in the lower jaw only of hemizygotes.

The picture formed is therefore one of a generalized partial suppression of growth and differentiation of dental epithelium with occasional localized points of abnormal overgrowth. The greatest variation was found in the lower molars. These will now be considered in more detail in the light of what is known of the development of the coat, and what has been observed in the fully formed dentition. A diagrammatic representation of the developmental sequence of relationships of the teeth of the lower jaw and the phases of hair follicle suppression is shown in Fig. 8.

The first period of hair follicle suppression, from 12½ to 17 days, is just that during which the first molar develops from a small bud of epithelium to an early stage of morphodifferentiation and histodifferentiation. At 17 days, the end of this suppression phase and the beginning of the phase of follicle formation, definite signs of overgrowth of the anterior extension of dental lamina were observed (Fig. 2E, G). At 19 days, towards the end of the follicle formation phase, the overgrowth had, in some instances, developed into a tooth germ in which histodifferentiation was almost as advanced, if not equally advanced, as in the first molar posterior to it (Fig. 3D, G). Subsequently, the various stages of histodifferentiation appeared to proceed together in the supernumerary and first molar germs.

2. *Stabilization of length of the tooth row*

The interpretation offered for these observations is based on the premise that there is a tendency for the length of the tooth row to be stabilized. Because of its retarded growth the first molar fails to occupy all the space allotted to it. As a consequence there is overgrowth of the dental lamina to form an additional tooth germ to take up the vacant space.

Perhaps it would be better to say that, at least during the developmental phase, the dental lamina is under a growth pressure which is inhibited when normally developing tooth germs have become established. Poor growth of developing germs would then result in a lesser degree of laminal inhibition which, if below a critical level, would allow the lamina to proliferate further. Such an explanation is consistent with the hypothesis that developing organs specifically inhibit like differentiation of the surrounding tissues (Rose, 1952, 1957). Evidence in support of this hypothesis has been provided by Saetren (1956) and by Clarke & McCallion (1959*a*, *b*).

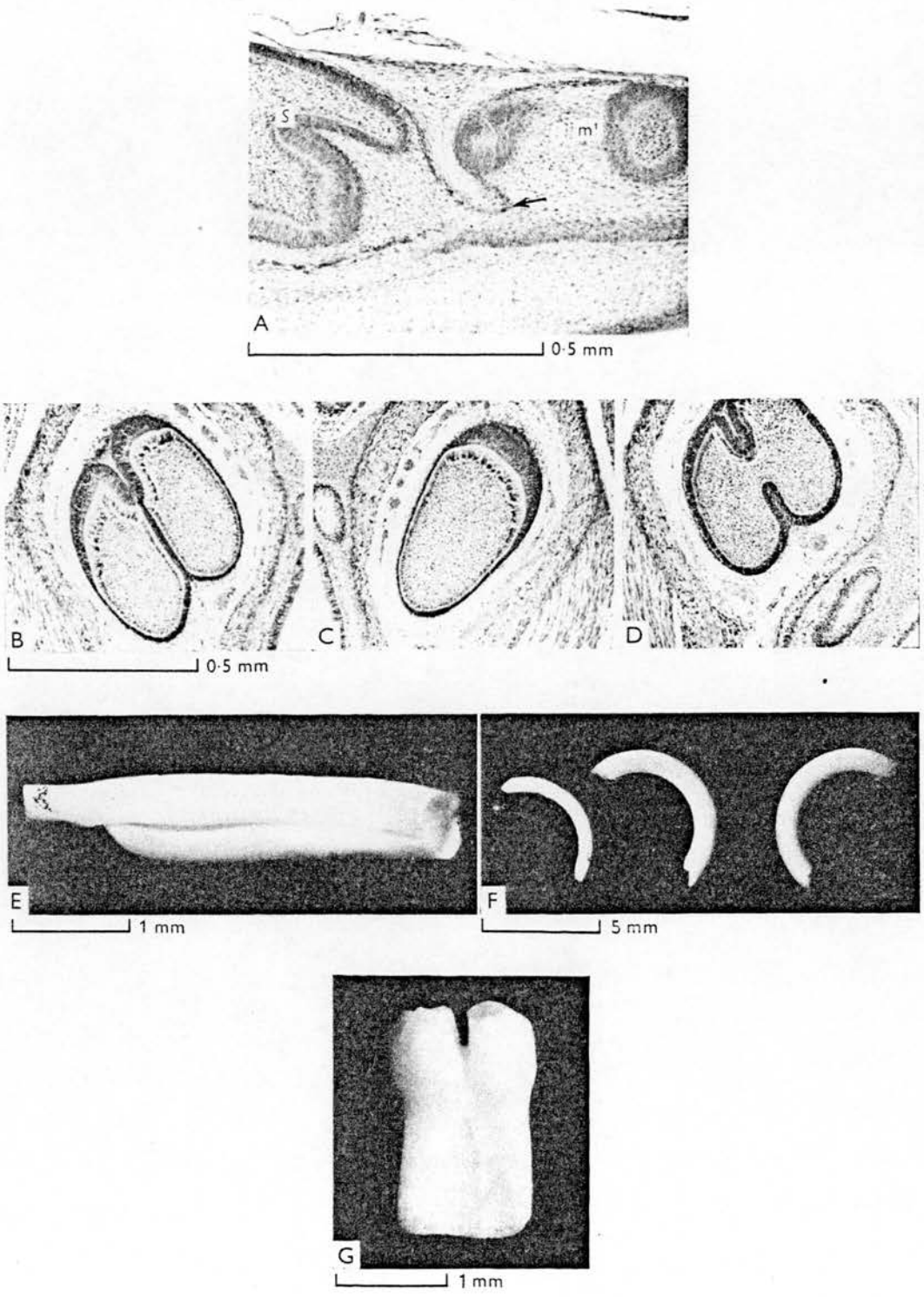
The formation of a supernumerary tooth at the anterior end of the molar row can therefore be regarded as a positive reaction to the small size of the

developing tooth row, tending to restore it to its normal length. There is good evidence that in the reverse situation a negative response can also occur, namely the reduction in size and eventual complete suppression of the third molar with increasing size of the first two (Grüneberg, 1951; Grewal, 1962; Van Valen, 1962). Van Valen (1962) also cites evidence in favour of the existence of such size interactions in a number of different developing systems.

The first obvious signs of laminal overgrowth were observed at 17 days—the end of the suppression phase and the beginning of the follicle formation phase. During the follicle formation phase rapid development of the supernumerary germ occurred. It is difficult to avoid the conclusion that this reaction was a response to relaxation of suppression. Such relaxation would no doubt affect the first molar as well as the dental lamina, and this could explain why epithelial downgrowths which failed to produce supernumerary germs were found. A sudden increase in size of the first molar germ could presumably prevent a downgrowth from developing further. However, there must be some difference in sensitivity to the suppressive influence between the laminal cells and those of the first molar germ. If there were not, no supernumerary downgrowths would develop. It is suggested that this difference in sensitivity is associated with the degree of differentiation of the two groups of cells, the less well differentiated cells of the dental lamina being more ready to react by proliferation as relaxation of suppression becomes more complete. The fact that the differentiating internal enamel epithelium is the first tissue to suffer degeneration as abnormality of the lower incisors increases, is evidence in favour of such a differential sensitivity.

The second molars, also in a less differentiated state than the first, would similarly be likely to react more readily to a relaxation of suppression. This would then be the basis of the general size interaction observed between first and second molars, especially noticeable in cases where no supernumerary was present. More specifically, it would explain the origin of the rampart of m^2. The existence of these size relationships in the fully formed dentition was recognized by Grüneberg, (1965).

If this interpretation of the observations is correct, then it is basically the size of the developing first molar at and before 17 days which controls the ultimate form of the whole molar row. The final size of the first molar is not a good indication of its status at 17 days, as recovery or further suppression could take place after 17 days and before its final form is decided by the onset of hard tissue formation. A slight difference in size between left and right first molars at 17 days could result in the successful formation of a supernumerary tooth germ on one side but the suppression of a potential counterpart on the other. Thus small differences in local conditions at a critical stage of development could be responsible for formidable asymmetry in the adult dentition.


S
m'
A
0·5 mm
B
C
D
0·5 mm
E
1 mm
F
5 mm
G
1 mm

3. *Incomplete twinning*

'Overt twinning' and 'concealed twinning' have already been discussed but no explanation has so far been given for 'incomplete twinning'. If the extra teeth found in the first two cases arise independently, then in the third the rare composite teeth observed must be the consequence of fusion rather than of incomplete fission. Hitchin & Morris (1966) showed that fusion of the developing incisors of the dog, or connation as they called it, is related to the persistence of dental lamina between two adjacent incisor germs. Rapid growth of adjacent germs was thought to cause the external enamel epithelium to be stripped off the persisting interdental lamina. As a result, the stellate reticulum of the two germs becomes confluent, their internal enamel epithelia come into contact, and fusion takes place. In addition to connation of two incisors of the normal series there were examples of connation of a first incisor with a supernumerary tooth. Figure 9A shows a case already illustrated in a different section (Fig. 5E). The external enamel epithelium between the supernumerary and first molar has just become separated from the underlying dental lamina. This illustration is comparable with one of those of Hitchin & Morris, though in their case separation was more extreme.

It seems probable that separation of the external enamel epithelium from persisting interdental lamina would not only be a function of rapid growth of adjacent tooth germs, but also of their proximity. The more tightly squeezed together the developing germs the greater the likelihood of epithelial stripping. Fusion would then be more likely to occur in the presence of a supernumerary tooth, as a greater than normal number of germs are then growing and competing for room in a restricted space.

In trials made prior to the main investigation some of the material was sectioned transversely. Figure 9B–D are of a tabby hemizygote at 21 days.

FIGURE 9

A. Tabby heterozygote at 19 days (the same example as in Fig. 5E, in a different section). The external enamel epithelium between the supernumerary and first molar germs is becoming separated from the underlying lamina (indicated by the arrow).

B. Tabby hemizygote at 21 days. Transverse section through the upper right incisor region. There are two germs with their internal enamel epithelia in intimate contact.

C. The left side of the same animal as in B, showing a single incisor germ.

D. The same example and the same side as in B, but further posteriorly to show a connexion between the pulp cavities of the two germs.

E. A fully formed composite upper right incisor from a tabby homozygote at 4 weeks of age.

F. Complete separation between a supernumerary and an upper right incisor in a tabby hemizygote, viewed from the buccal surfaces.

G. Lingual view of a composite lower right first molar from a downless homozygote.

Anteriorly on the left of the upper jaw there was a single normal incisor germ (Fig. 9C). Anteriorly on the right there were two incisor germs with their internal enamel epithelia in intimate contact (Fig. 9B). Further posteriorly on the right there was a connexion between the future pulp cavities of the two germs (Fig. 9D). The anterior end of a developing incisor is the first to form, whereas the posterior end is the youngest region where proliferation continues throughout life. The case just described must therefore have started out as two separate germs which fused subsequently. An example of a fully formed upper incisor of this sort is shown in Fig. 9E. It can be appreciated that once such a tooth has been subjected to wear the nature of its origin would be obscured. Figure 9F shows a case where separation between supernumerary and normal incisor has been maintained.

A similar argument can be used to explain the origin of composite molars with separate crowns and common roots. The crown develops before the root, so if the crowns are separate and the root common there must originally have been two germs which fused after formation of the crowns was complete. Such a case is illustrated in Fig. 9G.

Further evidence for the origin of fusion being associated with restriction of space comes from the study of artificially induced malformations. Knudsen (1965*a*, *b*, 1966 *a*, *b*) made a detailed study of the dental malformations associated with exencephaly induced in mice by teratogenic agents. There were various degrees of fusion of the two incisors within each jaw, and also intermediate cases where the future pulp cavities of the two germs were separate but their stellate reticulum was confluent. Upper incisor fusion was very much more common than lower incisor fusion. Ritter (1963) induced lower incisor fusion, and fusion of the lower molars of one side with those of the other, by *X*-radiation. These mandibular fusions were associated with mandibular micrognathy. Knudsen (1966*a*) reported on the molar malformations of exencephalic embryos. There were amazing cases of fusion of upper molar germs with lower molar germs on the same side. All these cases of fusion appear to have been associated with a reduction in the amount of connective tissue which normally separates the individual developing tooth germs.

The occurrence of fused and supernumerary molars in a less well known laboratory rodent, the rice rat, has been described briefly by Griffiths & Shaw (1961), and by Shaw, Griffiths & Osterholtz (1963). It may well be that the basis for these anomalies is similar to that discussed here for the tabby mouse.

SUMMARY

1. The development of the teeth of the tabby mouse has been studied and an attempt has been made to explain aspects of the dental abnormalities in terms of a single primary effect of the mutant gene, a partial suppression of the growth

and differentiation of dental epithelium. Such an explanation is consistent with the retarded growth and lack of differentiation of the coat.

2. It has been postulated that the level of this suppression varies in intensity at different stages of development in parallel with the observed effects on the developing hair follicles, and that the final outcome is dependent on an interplay of the suppressive influence and interaction between the developing teeth.

3. 'Twinning' of the lower molars was found to be due to the *de novo* development of a supernumerary tooth from a normal anterior extension of the dental lamina. Evidence for this in the upper molars was not complete, although observations here were not inconsistent with the lower jaw findings. There was no evidence of division of a first molar germ into two at any stage.

4. It seems most likely that the rare composite teeth observed in cases of 'incomplete twinning' are produced by fusion of the supernumerary with the adjacent germ of the normal series. Direct evidence for this was found in the upper incisors, and indirect evidence was found in upper and lower molars. Supporting evidence from other sources has been cited.

RÉSUMÉ

Aspects du syndrome 'tabby–crinkled–downless'.
I. Le développement des dents 'tabby'

1. Le développement des dents de souris '*tabby*' (tigré) a été étudié et on a tenté d'expliquer divers aspects des anomalies dentaires en termes d'un effet primaire unique du gène mutant, à savoir une suppression partielle de la croissance et de la différenciation de l'épithélium dentaire. Une telle explication s'accorde avec le retard de croissance et l'absence de différenciation du pelage.

2. On a postulé que le niveau de cette suppression varie en intensité à différents stades du développement, parallèlement aux effets observés sur les follicules pileux en cours de développement, et que le résultat final dépend d'une réaction réciproque de l'influence suppressive et de l'interaction entre les dents en cours de développement.

3. On a trouvé que la duplication des molaires inférieures était due au développement 'de novo' d'une dent surnuméraire à partir d'une expansion antérieure normale de la lame dentaire. La réalité de ce phénomène pour les molaires supérieures n'est pas évidente, quoique les observations faites ici ne soient pas en contradiction avec les résultats obtenus pour la mâchoire inférieure. Il n'est pas évident qu'un germe molaire primaire se soit divisé en deux à un stade quelconque.

4. Il paraît très vraisemblable que les rares dents composites observées dans les cas de duplication incomplète soient produites par fusion du germe surnuméraire avec le germe adjacent de la série normale. Une preuve directe de ceci a été trouvée pour les incisives supérieures, et une preuve indirecte l'a été pour les molaires supérieures et inférieures. On a cité les preuves à l'appui pour d'autres origines.

I am grateful to Professor D. S. Falconer for suggesting the investigation and for his interest and valuable advice during the work, to Professor C. H. Waddington for laboratory facilities, and to the Nuffield Foundation for financial support.

REFERENCES

CLARKE, R. B. & MCCALLION, D. J. (1959*a*). Specific inhibition of differentiation in the frog embryo by cell-free homogenates of adult tissues. *Can. J. Zool.* **37**, 129–31.

CLARKE, R. B. & MCCALLION, D. J. (1959*b*). Specific inhibition of neural differentiation in the chick embryo. *Can. J. Zool.* **37**, 133–6.

COHN, S. A. (1957). Development of the molar teeth in the albino mouse. *Am. J. Anat.* **101**, 295–310.

DUN, R. B. (1959). The development and growth of vibrissae in the house mouse with particular reference to the time of action of the tabby (*Ta*) and ragged (*Ra*) genes. *Aust. J. biol. Sci.* **13**, 312–30.

FALCONER, D. S. (1953). Total sex-linkage in the house mouse. *Z. indukt. Abstamm.-u. VererbLehre.* **85**, 210–19.

FALCONER, D. S., FRASER, A. S. & KING, J. W. B. (1951). The genetics and development of 'crinkled' a new mutant in the house mouse. *J. Genet.* **50**, 324–44.

GAUNT, W. A. (1955). The development of the molar pattern of the mouse (*Mus musculus*). *Acta anat.* **24**, 249–68.

GAUNT, W. A. (1956). The development of enamel and dentine on the molars of the mouse, with an account of the enamel-free areas. *Acta anat.* **28**, 111–34.

GAUNT, W. A. (1961). The development of the molar pattern of the golden hamster (*Mesocricetus auratus* W.), together with a re-assessment of the molar pattern of the mouse (*Mus musculus*). *Acta anat.* **45**, 219–51.

GREWAL, M. S. (1962). The development of an inherited tooth defect in the mouse. *J. Embryol. exp. Morph.* **10**, 202–11.

GRIFFITHS, D. & SHAW, J. H. (1961). Fused molars and supernumerary molars in the rice rat. *J. dent. Res.* **40**, 731–2.

GRÜNEBERG, H. (1943*a*). Congenital hydrocephalus in the mouse, a case of spurious pleiotropism. *J. Genet.* **45**, 1–21.

GRÜNEBERG, H. (1943*b*). The development of some external features in mouse embryos. *J. Hered.* **34**, 88–92.

GRÜNEBERG, H. (1951). The genetics of a tooth defect in the mouse. *Proc. R. Soc.* B **138**, 437–51.

GRÜNEBERG, H. (1965). Genes and genotypes affecting the teeth of the mouse. *J. Embryol. exp. Morph.* **14**, 137–59.

GRÜNEBERG, H. (1966*a*). The molars of the tabby mouse, and a test of the 'single-active *X*-chromosome' hypothesis. *J. Embryol. exp. Morph.* **15**, 223–44.

GRÜNEBERG, H. (1966*b*). More about the tabby mouse and about the Lyon hypothesis. *J. Embryol. exp. Morph.* **16**, 569–590.

HAY, M. F. (1961). The development *in vivo* and *in vitro* of the lower incisor and molars of the mouse. *Archs oral Biol.* **3**, 86–109.

HINRICHSEN, K. (1959). Morphologische Untersuchungen zur Topogenese der mandibularen Nagezähne der Maus. *Anat. Anz.* **107**, 55–74.

HITCHIN, A. D. & MORRIS, I. (1966). Geminated odontome—connation of the incisors in the dog—its etiology and ontogeny. *J. dent. Res.* **45**, 575–83.

KING, J. W. B. (1956). Linkage group *XIV* of the house mouse. *Nature, Lond.* **178**, 1126.

KNUDSEN, P. A. (1965*a*). Congenital malformations of upper incisors in exencephalic mouse embryos, induced by hypervitaminosis A. I. Types and frequency. *Acta odont. scand.* **23**, 71–90. II. Morphology of fused upper incisors. *Acta odont. scand.* **23**, 391–409.

KNUDSEN, P. A. (1965*b*). Fusion of upper incisors at bud or cap stage in mouse embryos with exencephaly induced by hypervitaminosis A. *Acta odont. scand.* **23**, 549–65.

KNUDSEN, P. A. (1966*a*). Congenital malformations of lower incisors and molars in exencephalic mouse embryos, induced by hypervitaminosis. A. *Acta odont. scand.* **24**, 55–90.

KNUDSEN, P. A. (1966*b*). Malformations of upper incisors in mouse embryos with exencephaly induced by trypan blue. *Acta odont. scand.* **24**, 647–77.

Mouse News Letter (1960). No. **23**, 30.

Mouse News Letter (1966). No. **34**, 32.

RITTER, W. (1963). Durch Röntgenstrahlen induzierte Zahnverschmelzungen bei der Maus. II. Mitteilung: Histologische Befunde. *Dt. Zahnärztl. Z.* **18**, 1063–8.

ROSE, S. M. (1952). A hierarchy of self-limiting reactions as the basis of cellular differentiation and growth control. *Am. Nat.* **86**, 337–54.

ROSE, S. M. (1957). Cellular interaction during differentiation. *Biol. Rev.* **32**, 351–82.

SAETREN, H. (1956). A principle of auto-regulation of growth. Production of organ specific mitosis-inhibitors in kidney and liver. *Expl Cell Res.* **11**, 229–32.

SHAW, J. H., GRIFFITHS, D. & OSTERHOLTZ, M. (1963). Relationship between body weight and occurrence of the fused molar and supernumerary molar traits in the rice rat. *Archs oral Biol.* **8**, 777–8.

SOFAER, J. A. (1969). Aspects of the Tabby-Crinkled-Downless Syndrome. II. Observations on the reaction to changes of genetic background. *J. Embryol. exp. Morph.* **22**, 207–27.

VAN VALEN, L. (1962). Growth fields in the dentition of *Peromyscus*. *Evolution* **16**, 272–8.

(*Manuscript received 2 December 1968*)

J. Embryol. exp. Morph. Vol. 22, 2, pp. 207–27, September 1969
Printed in Great Britain

Aspects of the tabby-crinkled-downless syndrome

II. Observations on the reaction to changes of genetic background

By J. A. SOFAER[1]

The Institute of Animal Genetics, West Mains Road, Edinburgh

Two alleles of the sex-linked gene tabby, *Ta* and *Ta*^{c}, and two autosomal mimics of tabby, crinkled, *cr*, and downless, *dl*, each produce a similar mutant syndrome involving the coat and dentition of the mouse. Certain aspects of the development of the mutant defects have already been studied, and some comparisons between tabby and crinkled have been made. The present investigation is concerned with a comparison of all four genes, and with further analysis of the developmental basis for the dental abnormalities.

As the expression of a mutant gene can be modified considerably by genetic background, critical comparison between genes with similar effects requires a common genetic environment. The four genes under consideration here were crossed twice from their stock backgrounds to two standard inbred strains. Conclusions were then drawn from the reactions of characters scored to background change, rather than from the absolute level of expression in any one background. Comparative information about the genes was derived from a consideration of the relative reaction of each character between genes, and inferences as to the developmental basis for some of the dental abnormalities were drawn from the relative reactions of different characters over all genes. In addition, a combination of within and between gene comparisons gave some indication of the level at which background modification was operating. Also, the wide range of expression of the syndrome produced by the four genes in different genetic backgrounds was itself useful in the developmental analysis.

MATERIAL AND METHOD

The superficial features of all mutant homozygotes and tabby hemizygotes include: abnormal texture of the coat, a reduced complement of facial vibrissae, a bald patch behind each ear, and, with the exception of *Ta*^{c} homozygotes and hemizygotes, a more or less bald tail which is sometimes kinked towards the tip. The tails of *Ta*^{c} homozygotes and hemizygotes are generally covered with hair

[1] *Author's address:* National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

and are rarely kinked. However, Ta^c tail hairs are not entirely normal and are more sparsely distributed than in the normal mouse. All homozygotes and tabby hemizygotes have characteristically reduced molars, and incisors and third molars are sometimes absent.

Crinkled and downless heterozygotes are generally outwardly normal, though there is sometimes a faint suggestion of the mutant phenotype. Tabby heterozygotes which are phenotypically agouti show varying intensities of transverse striping and are therefore immediately recognizable. They also show other definite signs of the mutant phenotype to a variable extent. The dentitions of all the heterozygotes may contain normal teeth, frankly mutant teeth, and teeth combining characteristics of both the normal and the mutant phenotypes. All three types of tooth may be present in the same animal. A further feature of the heterozygote dentition is the rare occurrence of an additional molar tooth.

Crinkled and both tabby alleles arose and have been maintained at the Institute of Animal Genetics, Edinburgh. Downless animals were obtained from the Radiobiological Research Unit, Harwell.

1. *The genes*

(i) *Crinkled*

The first crinkled mouse appeared in the progeny of a male treated with nitrogen mustard (Auerbach & Falconer, 1949). The genetics of the crinkled gene and the development of abnormalities produced by it were studied by Falconer, Fraser & King (1951). Crinkled has been assigned to linkage group XIV (King, 1956). The teeth of crinkled mice have been described by Grüneberg (1965, 1966).

(ii) *Tabby*

The original tabby allele, here called Ta^F, arose in a strain selected for large size on a low plane of nutrition. The original tabby male was at first thought to be a crinkled mouse, but the new mutation subsequently proved to be sex-linked (Falconer, 1953). The structure of the tabby coat was indistinguishable from that of crinkled and its development was presumed to be the same. The teeth of tabby (Ta^F) mice have been described by Grüneberg (1965, 1966), and their development has been investigated by Sofaer (1969).

Ta^c arose in a line selected for body weight (*Mouse News Letter*, 1966*b*). Ta^c resembles another tabby allele of independent origin, Ta^j (*Mouse News Letter*, 1963).

(iii) *Downless*

Downless first appeared as an independent autosomal mutation resembling crinkled. Crossing with a crinkled mouse showed that the new mutation was not at the crinkled locus (*Mouse News Letter*, 1960). Downless has been assigned to linkage group IV (*Mouse News Letter*, 1966*a*).

2. *Genetic background*

Isaacson (quoted by Grüneberg, 1965) found that the incidence of incisor abnormality in tabby mice was influenced by genetic background. Homozygous tabby females from stock were crossed to a number of inbred strains and the F_1 tabby males were examined. The two most extreme degrees of manifestation were 85·5 % abnormal in the *A* strain F_1, and 11·0 % abnormal in the *JU* F_1. Strains *A* and *JU* were consequently chosen as the backgrounds on which the genes were to be compared. Both these strains have been maintained by brother × sister mating at the Institute of Animal Genetics, Edinburgh, for over forty generations.

The main body of the material examined was composed of five classes of animals according to background genotype: the stock background; the backgrounds after one cross to *JU* ($\frac{1}{2}$ *JU*), and after two crosses to *JU* ($\frac{3}{4}$ *JU*); and the backgrounds after one cross to *A* ($\frac{1}{2}$ *A*), and after two crosses to *A* ($\frac{3}{4}$ *A*). All of these animals were the progeny of heterozygous mothers and homozygous or hemizygous fathers, and in each mating both parents had the same level of background genotype.

Within each background class there were four subclasses, one for each of the genes. Within each tabby subclass there were four groups according to genotype—*TaTa*, *Ta*+, *Ta*, and +—the wild-type males of the two tabby stocks being designated +(Ta^F) and +(Ta^c). Within each crinkled and downless subclass there were two groups, one of homozygotes and one of heterozygotes, in which the sexes were balanced as far as possible. An average of six matings was made within each subclass, and in most cases material was drawn from several litters of each mating. Unfortunately the two subclasses tabby (Ta^F) $\frac{3}{4}$*JU* and downless $\frac{3}{4}$ *JU* were not completed because of poor fertility. There were two additional groups, one of *A* strain and one of *JU* strain control animals. The number of individuals in each group varied between 21 and 26, and in all about 1300 animals were examined.

3. *Characters scored*

(i) *Vibrissa number*

On each side of the face of the normal mouse there are two supraorbital, one postorbital, and two postoral vibrissae. Beneath the chin is a single group of three inter-ramals. The supraorbital, postorbital and postoral vibrissae of both sides are called groups A, B, and C respectively, and the inter-ramals are called group D.

A survey by Dun (1958) and additional data of Dun & Fraser (1959), together comprising about 6000 mice from a variety of stocks, showed that variation in vibrissa number is normally very limited. Most of the variability that was present was restricted to group D, which occasionally had two rather than three vibrissae. Group B was absolutely invariant. The variation of inter-ramal score was at first thought to be due to sensitivity of this group to environmental fluctuation.

However, crossing inbred strains which differed in respect of their inter-ramal score produced an intermediate F_1, and selection for high and low inter-ramal score was effective in both directions (Dun, 1958). The variation was thus shown to be genetic in nature.

Reduction in the number of secondary vibrissae in crinkled and tabby mice was first noticed by Falconer *et al.* (1951) and Falconer (1953). Not only is the total number reduced, but variability is increased (Fraser, Nay & Kindred, 1959), and this variation responds to selection (Dun & Fraser, 1959).

Groups A–D were all scored in the present study.

(ii) *Tabby heterozygote striping*

The intensity of tabby heterozygote striping has been shown to respond to selection for vibrissa number (Dun, 1959). The degree of striping can therefore be modified by genetic background. Animals were scored on a six-point scale where zero was indistinguishable from wild-type and 5 was the maximum intensity of striping.

(iii) *The dentition*

(*a*) *The level and pattern of abnormality.* Twenty-four characters within the dentition were considered. The object of the scoring method was to obtain an estimate of the mean level of abnormality of each character in each group of animals. The method combined an assessment of both penetrance and expressivity. Table 1 lists the characters and shows the scores given to the different degrees of expression of each one. No intermediate level of expression was scored for characters, 8, 9, 17, 18, 22, 23 and 24. Both sides were considered together, so that the maximum possible score was 4 per character per mouse. In each group of animals the total observed score for each character was expressed as a percentage of the total possible maximum. The effects of the mutant genes on the dentition were therefore considered in terms of percentage abnormality. A joint consideration of all the characters taken individually indicated the pattern as well as the level of abnormality.

(*b*) *The incidence of lower supernumerary teeth.* The rare occurrence of additional molar teeth in tabby and crinkled heterozygotes was recognized by Grüneberg (1966), who suspected that such additional teeth also occurred in homozygotes and tabby hemizygotes. This has indeed been shown to be the case (Sofaer, 1969). Because of the relatively low incidence of upper supernumerary teeth, only lower supernumeraries are considered here.

The diagnosis of lower supernumerary teeth in adult heterozygotes is usually a relatively simple matter. Comparison of all the molar teeth on the affected side, of the affected side with the opposite side, which is usually more normal, and of the affected animal with other animals, leaves little room for doubt. In homozygotes and hemizygotes diagnosis is a little more difficult. Extreme reduction of all the lower molars is the general rule so that morphological criteria

are of little use. In this study the sole criterion adopted for scoring supernumeraries in homozygotes and hemizygotes was relative size of the first two standing molars. If the first was smaller than the second it was regarded as a supernumerary. This, however, is not always the case. Sofaer (1969) reported an instance where the tooth taken to be m_1 on the basis of its developmental state was much smaller than m_2. Nevertheless, this criterion was considered to be the most appropriate one.

Table 1. *The scoring of twenty-four characters within the dentition*

Ra = rampart, Rt = number of roots, N = normal, R = reduced, A = absent, P = present, L = enlarged.

	Character scored on each side		Score 0	1	2
1	Upper incisor		N	R	A
2	Lower incisor		N	R	A
3	m^1	B1	N	R	A
4		B3	N	R	A
5		1–L1 separation	N	R	A
6		L1–L2 separation	N	R	A
7		Rt	3	2	1
8	m^2	Ra	P	—	A
9		B1	N	—	L
10		B3	N	R	A
11		Rt	3	2	1
12	m_1	L1	N	R	A
13		B1	N	R	A
14		B2–L2 separation	N	R	A
15		B3–L3 separation	N	R	A
16		4	N	R	A
17		Rt	2	—	1
18	m_2	B1	P	—	A
19		B2–L2 separation	N	R	A
20		B3–L3 separation	N	R	A
21		4	N	R	A
22		Rt	2	—	1
23	Upper third molar		P	—	A
24	Lower third molar		P	—	A

4. *Procedure*

Animals were collected at 4 weeks of age, by which time all the teeth are fully formed, except that root length has not yet reached a maximum. Vibrissa number and tabby heterozygote striping were scored immediately after sacrifice. The animals were then decapitated and the heads skinned and prepared by the method of Luther (1949). The dentitions were stored and examined subse-

quently. The counting of the vibrissae and examination of the dentitions were carried out under a dissecting microscope.

In some of the previous work the abnormal postorbital vibrissae, detectable in tabby animals 5 days after birth, were scored (Dun, 1959). These fibres are lost with the growth of the coat. As scoring in the present study was at 4 weeks of age only vibrissae with detectable sinus hair follicles were recorded.

RESULTS

1. *Vibrissa number*

The two inbred strains used in the present study, *A* and *JU*, differed in respect of both the C and D vibrissa groups. The effect of crosses between the two strains on the scores at these two sites is shown in Fig. 1. In each parental

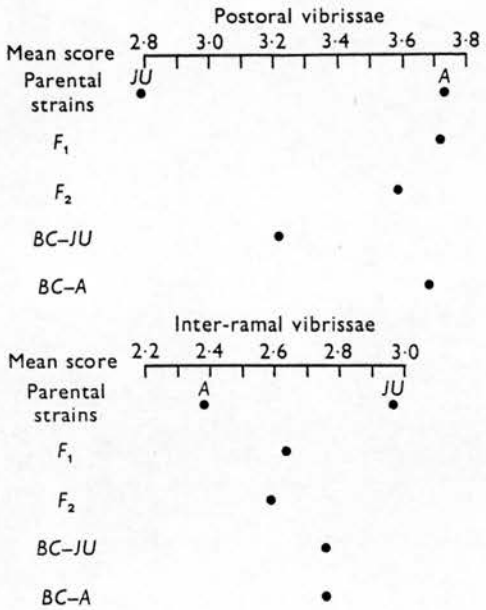


Fig. 1. The effect of crosses between strains *A* and *JU* on postoral and inter-ramal vibrissa scores.

strain there was a high score at one site and a low score at the other. High score or low score is therefore not necessarily a property common to both groups together. In group C high score appeared to be almost completely dominant over low score, whereas in group D high score and low score had about equal weight. These observations suggest that the two groups are to some degree under separate genetic control. Other indications of the existence of separate genetic systems influencing the different groups have been reported by Fraser *et al* (1959) and by Fraser & Kindred (1962).

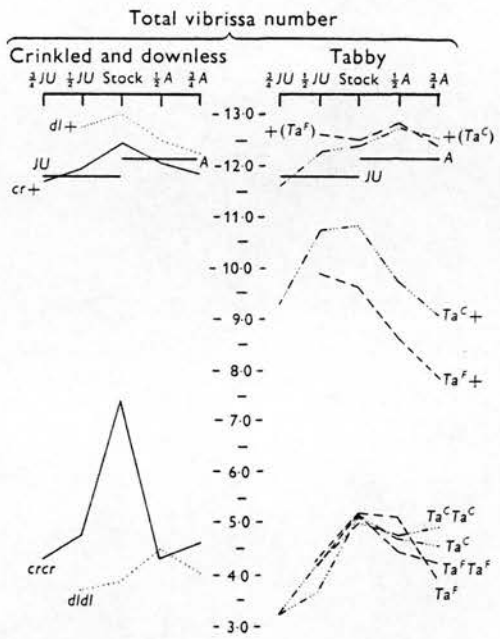


Fig. 2

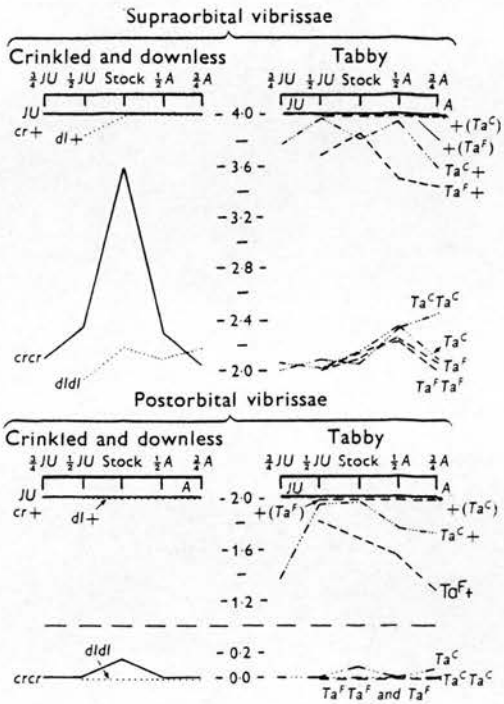


Fig. 3

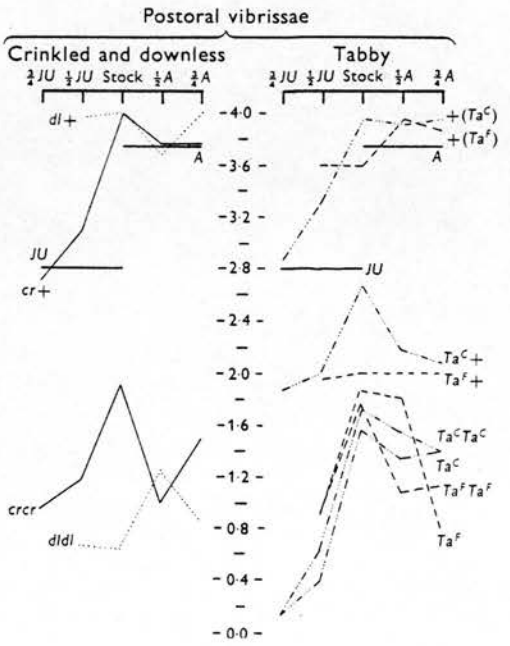


Fig. 4

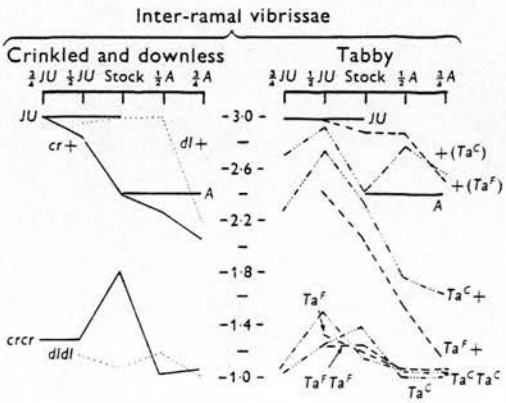


Fig. 5

Figs. 2–5. The reaction of vibrissa number to background change.

The variation of vibrissa number in mutant animals with changes in genetic background is presented diagrammatically in Figs. 2–5, first for total vibrissa number, then for each group separately. Each point represents the mean vibrissa score for that group of animals. The mean levels of the *A* and *JU* pure strain groups are shown as heavy horizontal lines. Throughout, crinkled and downless are compared with the two tabby alleles.

Both crinkled and downless behaved as more or less complete recessives. Although there was variation, the heterozygote levels did not fall much below those of the *A* and *JU* strains in any of the vibrissa groups. The greatest deviation from complete recessivity was shown in group D after two crosses to the *A* strain. By contrast, both tabby alleles showed considerable variation in dominance, both between vibrissa groups and as a reaction to background change. Dominance was least in the supraorbital and postorbital groups. In the postoral group heterozygotes were intermediate and relatively stable compared with their wild-type and mutant litter-mates. In the inter-ramal group heterozygotes showed the widest variation, whereas their wild-type and mutant litter-mates were relatively insensitive to background change. Dominance differences between the different vibrissa groups have been previously reported for tabby (Ta^F) by Fraser *et al.* (1959).

Fraser *et al* (1959) also suggested that differences between stock tabby (Ta^F) and crinkled mice were due to background genotype. The present results are consistent with this suggestion as crinkled homozygotes, which had a high score in all groups on the stock background, tended to drop towards a common homozygote–hemizygote level when crossed to the inbred strains. In addition, Kindred (1967) crossed crinkled into the stocks that had been selected for high and low vibrissa number in tabby. The extreme phenotypes of tabby (Ta^F) and crinkled were found to be identical. The present results provide no evidence to suggest that the extreme phenotypes of any of the genes differ to any appreciable extent.

2. *Tabby heterozygote striping*

Unfortunately both the inbred strains used were albino. The consequent small proportion of phenotypically agouti animals in the crosses made it necessary to pool the $\frac{1}{2}$ and $\frac{3}{4}$ groups of each background. No zero-grade animals were found. The mean scores of the stock and pooled backgrounds are shown in Fig. 6, and the numbers of animals that contributed to each mean are shown in parentheses. Although there was a marked difference in striping score between the two alleles on their stock backgrounds, the difference became reduced after crossing. This therefore suggests a fundamental similarity between the two alleles with respect to striping score.

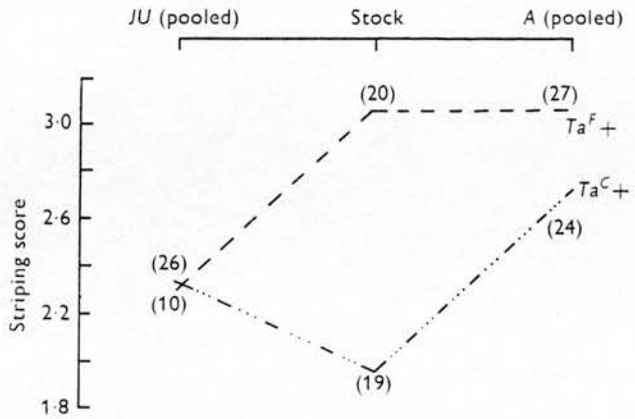


Fig. 6. The reaction of tabby heterozygote striping to background change.

3. *The dentition*

(*a*) *Level and pattern of abnormality*

Grüneberg (1966) has already commented on the non-random involvement of the different regions of the dentitions of tabby and crinkled heterozygotes. The present results show that a pattern of abnormality was generally well maintained within heterozygotes at different levels of expression produced by different genetic backgrounds. Basically the same pattern was shown by homozygotes and hemizygotes. The results are expressed diagrammatically according to the scoring system described.

Ta^F+ heterozygotes (Fig. 7) showed a wide range of expression and the pattern was well maintained throughout. The stock background was intermediate, and expression was increased by crossing to the *A* strain and decreased by crossing to *JU*. Ta^c+ heterozygotes (Fig. 8) did not show the same range of expression and this was presumably associated with low penetrance on the stock background. Crossing to *JU* produced a slight increase of abnormality, whereas crossing to *A* increased it to a level approaching that shown by Ta^F+ animals on the *A* strain background. Thus, although the expression of Ta^c+ and Ta^F+ on their stock backgrounds was very different, the difference was reduced by making genetic background more common to both.

Both *cr*+ and *dl*+ heterozygotes showed relatively low penetrance of the abnormalities and little variation with change of genetic background (Fig. 9). The lower jaw was hardly affected at all. In the upper jaw the level of expression of *cr*+ on the stock background was higher than that of Ta^c+ on its stock background. However, whereas crossing to the *A* strain markedly increased the level of abnormality shown by Ta^c+, abnormality was reduced in *cr*+ animals. Downless behaved in a similar way.

The control groups are presented in Fig. 10 for comparison. There were slight deviations from total normality. The low frequencies of reduction in size of

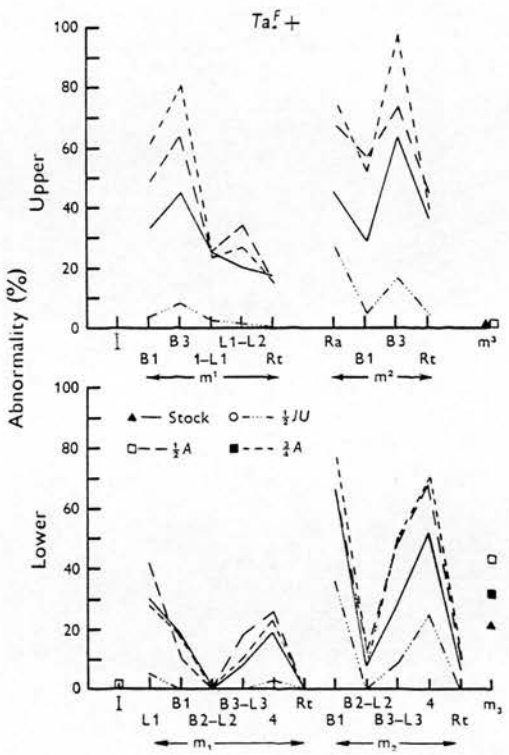


Fig. 7

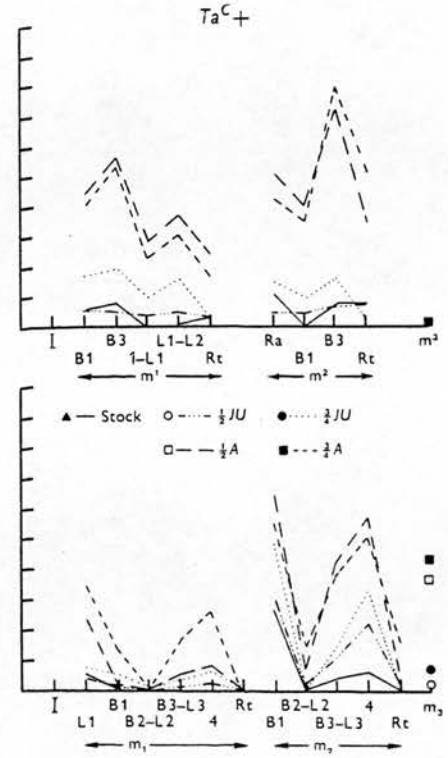


Fig. 8

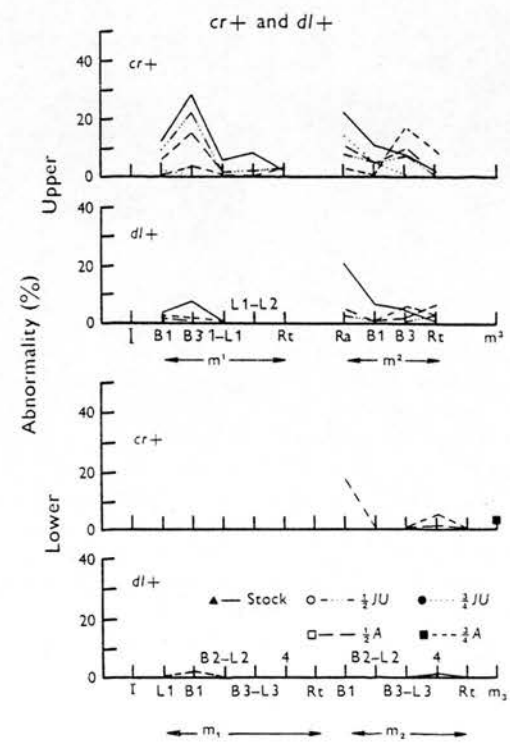


Fig. 9

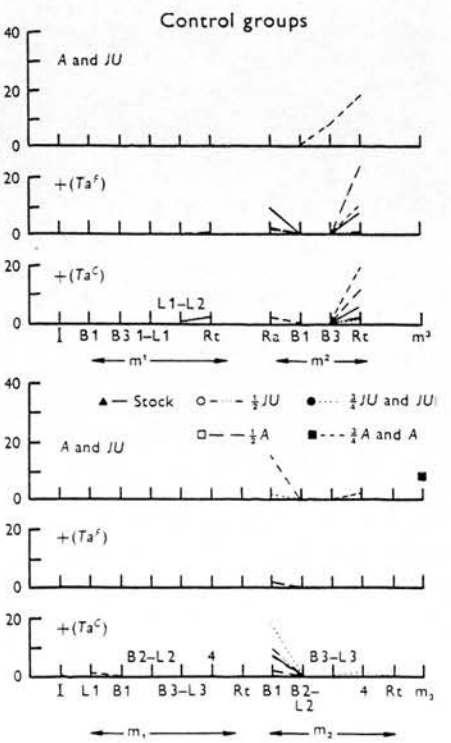


Fig. 10

Figs. 7–10. Variation in level of abnormality within the dentition with background change.

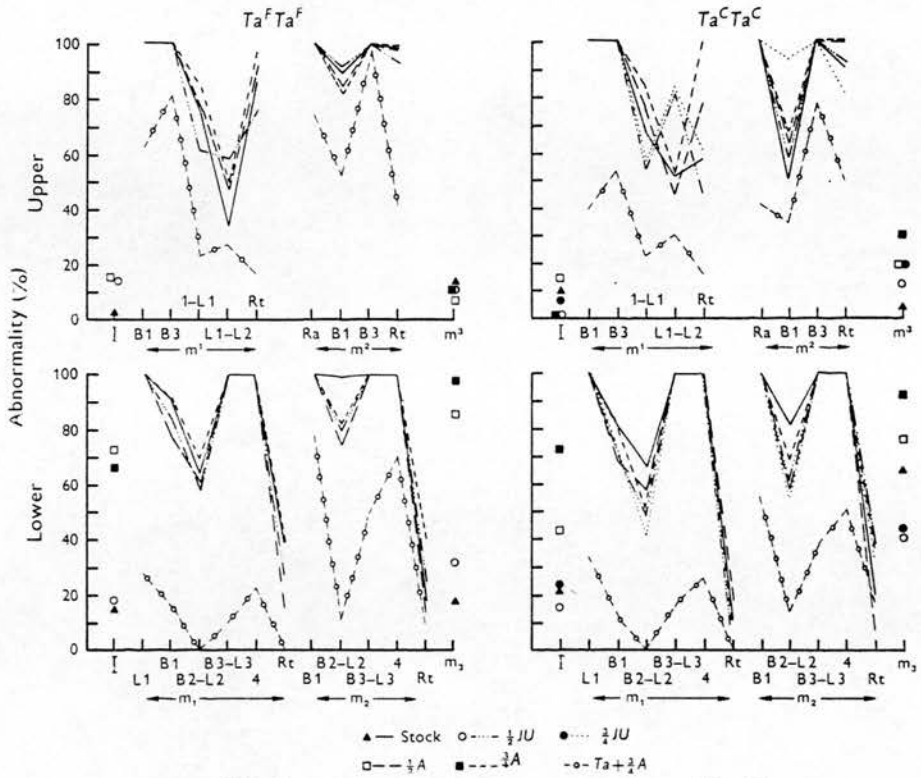


Fig. 11 Fig. 12

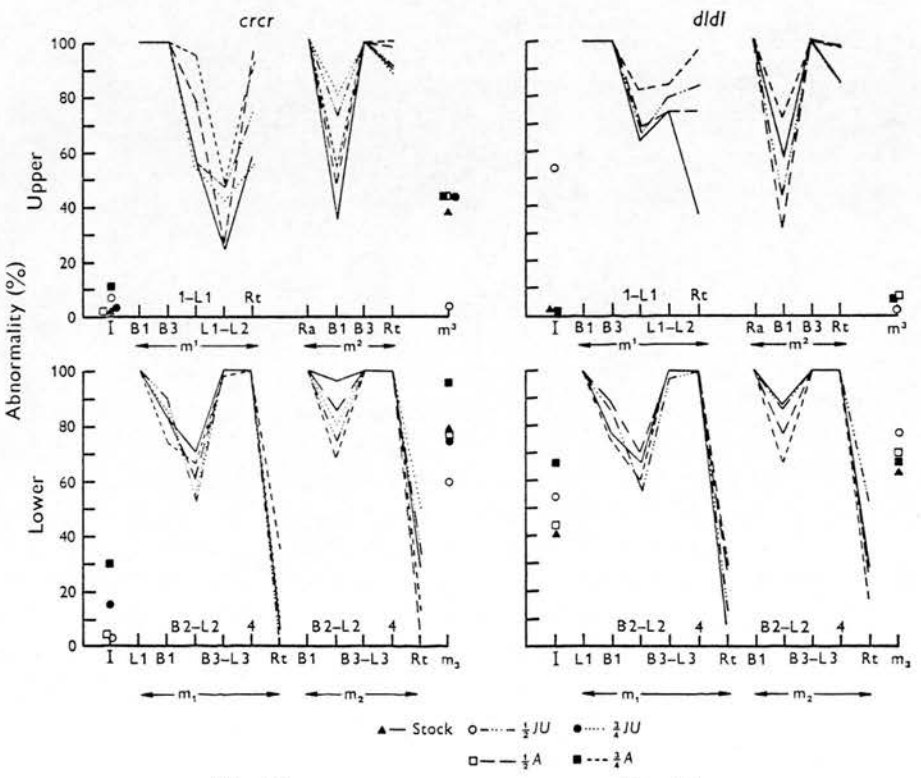


Fig. 13 Fig. 14

Figs. 11–14. Variation in level of abnormality within the dentition with background change.

cusps and number of roots, and the absence of third molars in the *A* strain, were not unexpected. However, the occasional occurrence of a rampart in the $+(Ta^F)$ and $+(Ta^c)$ groups was surprising, as this characteristic was thought to be due specifically to the direct influence of the mutant genes. The *A* strain, which as a background favoured the production of abnormalities in mutant animals, itself showed the highest mean incidence of abnormality amongst the control groups.

In homozygotes the range of expression of abnormality shown by first and second molars was much less than in heterozygotes. That shown by incisors and third molars was increased. In Figs. 11 and 12 the levels of the $Ta^F + \frac{3}{4}A$ and $Ta^c + \frac{3}{4}A$ groups for first and second molars are shown in conjunction with the homozygote results so that homozygotes and heterozygotes can be compared. The only inconsistency in the pattern between homozygotes and heterozygotes involves cusps L 1 and L 2 of m^1. In the Ta^cTa^c group, and to a lesser extent in the Ta^FTa^F and *crcr* groups also, there was a difference of pattern in m^1 between animals with *A* and *JU* backgrounds. The general level and pattern of expression shown by the *crcr* group (Fig. 13) were similar to those shown by tabby. The *dldl* group differed slightly from the others, but only in respect of the pattern and intensity of abnormality in m^1 (Fig. 14). Tabby hemizygotes were no different from their respective homozygous litter mates.

Total tooth score, the total mean score of all twenty-four characters and an indication of the degree of abnormality of the dentition as a whole, is shown in Fig. 15. As with vibrissa score the picture is one of near recessivity of crinkled and downless and intermediate dominance of tabby. In crinkled and downless heterozygotes, and in all homozygotes and hemizygotes, expression was relatively stable. Tabby heterozygotes showed a relatively wide range of variation with background genotype.

(*b*) *Incidence of lower supernumerary teeth*

Figure 16 shows the incidence of lower supernumeraries in the different background groups of animals, heterozygotes being separated from homozygotes and hemizygotes. The number of lower quadrants with supernumeraries was expressed as a percentage of the total for each group. There are two very definite opposing trends. In tabby heterozygotes the *A* strain background favoured the development of supernumeraries, whereas in homozygotes and hemizygotes the situation was completely reversed.

4. *Correlated responses*

Previous work has shown that selection for total vibrissa number produces correlated responses in other aspects of the tabby phenotype. Dun (1959) demonstrated a negative correlation between vibrissa number and tabby heterozygote striping. Dun & Fraser (1959) found that the tabby gene depressed growth

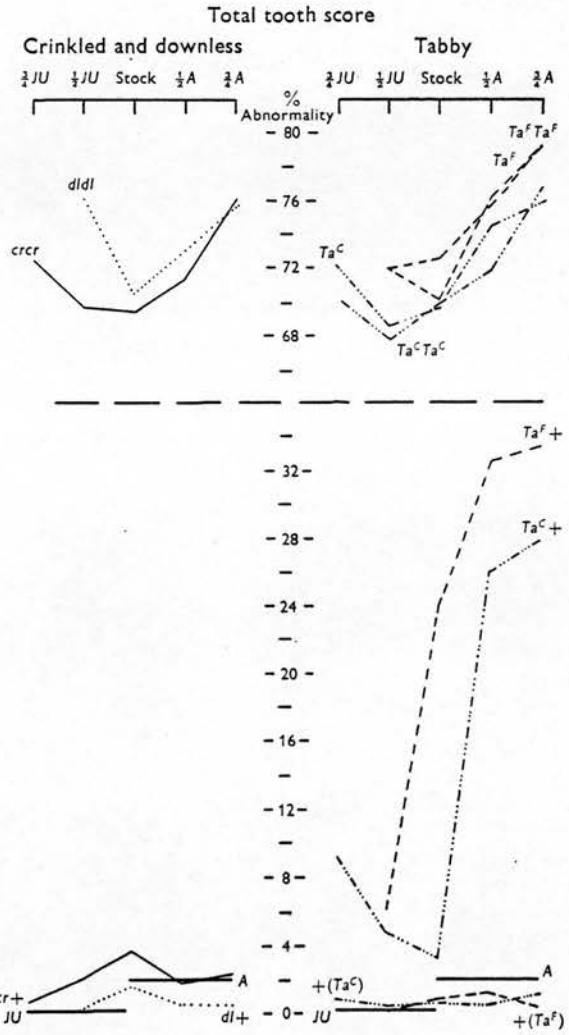


Fig. 15. The reaction of abnormality of the dentition as a whole to background change.

more markedly in the low selection line than in the high selection line. Fraser & Kindred (1962) showed that the number of mystacial vibrissae in tabby mice, which is invariant in unselected stocks, responded slightly to selection practiced on the secondaries (groups A–D plus the ulnar-carpals).

The relative responses to changes of genetic background of the following characters scored in the present study will now be considered: total vibrissa number and tabby heterozygote striping; total vibrissa number and total tooth score; total tooth score and the incidence of lower supernumerary teeth.

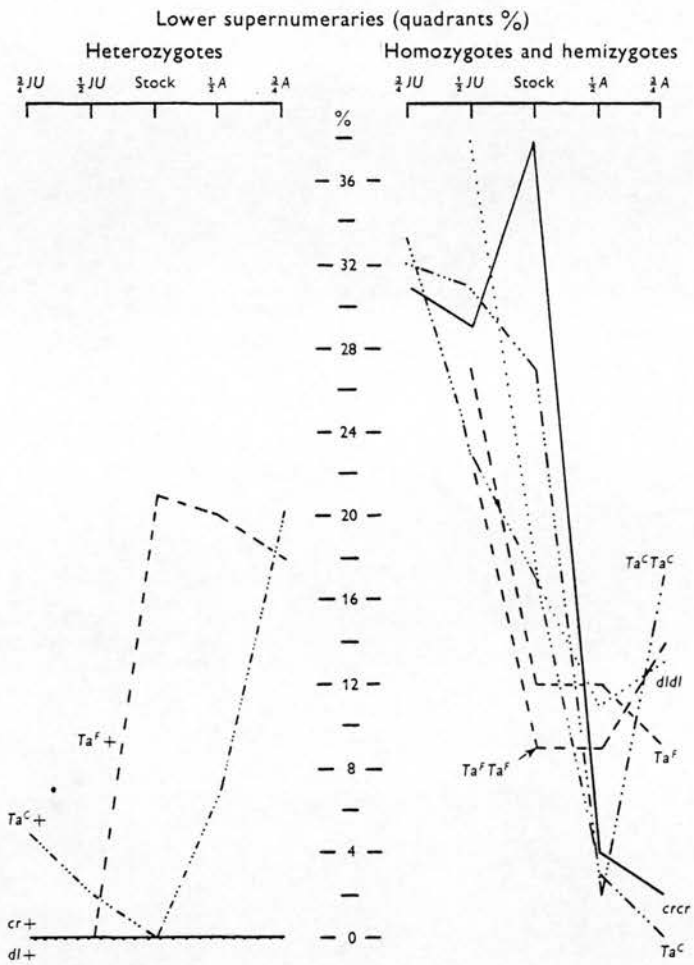


Fig. 16. Variation in the incidence of lower supernumerary teeth with background change.

(i) *Total vibrissa number and tabby heterozygote striping*

The total vibrissa scores of heterozygotes of the $\frac{1}{2}$ and $\frac{3}{4}$ groups of each background were pooled and compared with the mean striping scores of these groups. There was a high negative correlation ($r = -0{\cdot}85$; $P < 0{\cdot}05$).

(ii) *Total vibrissa number and total tooth score*

Tabby heterozygotes were considered separately from all homozygotes and hemizygotes. Crinkled and downless heterozygotes were not included in the analysis. Correlation coefficients were calculated from the mean scores of each group of animals; there were therefore nine pairs of measurements for tabby heterozygotes, and twenty-seven pairs for homozygotes and hemizygotes of all the genes.

A high negative correlation was found between total vibrissa number and total tooth score amongst tabby heterozygotes ($r = -0{\cdot}83$, $P < 0{\cdot}01$), but amongst homozygotes and hemizygotes there was only a very low non-significant negative correlation ($r = -0{\cdot}15$). This discrepancy seems to be largely attributable to the reducing effect of the *JU* background on group C vibrissae. The effect was marked in tabby homozygotes and hemizygotes, but not in heterozygotes (Fig. 4). As the *JU* background favoured greater normality of the teeth, the vibrissa effect must have tended to blur any inverse relationship between total tooth score and vibrissa number.

(iii) *Total tooth score and lower supernumeraries*

Correlation coefficients were calculated as described above. There was a high positive correlation between total tooth score and the incidence of lower supernumeraries amongst tabby heterozygotes ($r = +0{\cdot}89$, $P < 0{\cdot}01$), but a negative correlation amongst homozygotes and hemizygotes ($r = -0{\cdot}45$, $P < 0{\cdot}05$). These figures reflect the opposing trends already illustrated in Fig. 16.

The positive correlation amongst heterozygotes is to some extent spurious in that interaction between supernumerary and first molar occurs. The presence of a supernumerary would be automatically associated with an abnormal m_1, and probably a reduced m_2 and absent m_3. However, if m_1 were not intrinsically abnormal the supernumerary would probably never have developed. In homozygotes and hemizygotes the incidence of supernumeraries was highest in the least abnormal dentitions, that is, at the lowest level of mutant expression. There does not appear to be any reason why this relationship should be spurious, as m_1 is more likely to be reduced to a point where m_2 is larger, a condition where m_1 could be mistaken for a supernumerary, in the most abnormal dentitions. This kind of misdiagnosis of supernumeraries would therefore tend to obliterate a negative correlation. The embryological evidence suggests that a supernumerary could only rarely be larger than the first molar it precedes, so that the reverse situation, namely mistaking a supernumerary for a first molar, is unlikely to occur unless both teeth are of a similar size. As there were relatively few cases where the first two standing molars were of a similar size, misdiagnosis of this type is not likely to provide a major source of error. It is thus concluded that both correlation coefficients reflect the true nature of the situation.

DISCUSSION

1. *The pattern of abnormality in the dentition*

In a study of tabby (Ta^F) and crinkled, Grüneberg (1966) showed that the mixed features of the tabby heterozygote dentition cannot be accounted for in terms of cell specific mosaicism. The present study, with the added dimension of variation in the background genotype and including Ta^c and downless, has reinforced this conclusion. Homozygotes and hemizygotes showed basically the

same pattern of abnormalities as heterozygotes in a more extreme form, and the pattern was well maintained at different levels of expression within each genotype group. The developmental basis for the pattern must therefore be common to all the genes at all levels of expression.

The areas which are abnormal in mutant teeth are generally those which develop late. The cusp pattern of a mutant molar may therefore be the result of a more or less normal but rather slow course of development which has been interrupted by the relatively premature onset of calcification. This interpretation can be tested by comparing the relative sensitivities to the mutant genes of the different regions of each tooth with the order in which these regions normally develop. The relative sensitivities can be derived from the data already presented.

Considering first m_1, the order of sensitivity, starting with the most sensitive region, was L1, 4, B3–L3, B1, B2–L2. This is almost exactly the reverse of the sequence in which these regions normally develop, the only qualification being that an anterior extension of the developing crown, which subsequently gives rise to cusp L1, is present before cusp 4 appears. The order in m_2 was B1, 4, B3–L3, B2–L2. This differs from m_1 in that B1 was the most sensitive region. However, cusp B1 of m_2 is very small, and is sometimes poorly defined and even absent in 'normal' mice. It is therefore not difficult to understand how it could be lost first with general reduction of size and complexity of the whole crown. The first two lower molars therefore fit the interpretation reasonably well.

An objection arises when the upper molars are considered in this way. Referring to Gaunt (1955, 1956, 1961), Grüneberg (1965) pointed out that cusp B3, shown by the present data to be the most sensitive region, develops early. It seems reasonable however to try and find an explanation for this discrepancy within the framework of the suggested interpretation, as no alternative explanation of the pattern is apparent at present.

The most obvious difference between normal and tabby m^1 germs at an early stage of morphogenesis is the greater bulbosity of the tabby germ (Sofaer, 1969). A comparison of wax reconstructions of control and *Ta* m^1 germs at 17 days of gestation showed that the tabby germ was not only considerably shorter anteroposteriorly, but also a good deal wider. This difference in shape may well be associated with a difference in the distribution of tensional forces within the internal enamel epithelium, a factor which has been considered to play a role in the establishment of crown pattern (Butler, 1956). A difference in the distribution of tension in the posterior part of the crown at a critical stage could, perhaps, prevent the formation of the sulcus which normally develops between B3 and the future cusp 3. If this were the case, what has all along been called cusp 3 of tabby upper molars would be homologous with a normal cusp B3. The proliferation that would normally have gone to producing a sulcus between B3 and cusp 3, and to enlarging the forming cusp 3, would then be diverted to

enlarging B3 alone. Cusp B3 could then take up a fairly central position at the posterior end of the crown.

The development of the rampart in tabby upper second molars starts at a relatively early stage of morphogenesis (Sofaer, 1969). The development of the rampart, thought to be an attempt to compensate for the small size of m^1, need not therefore be inconsistent with suppression of the late developing regions.

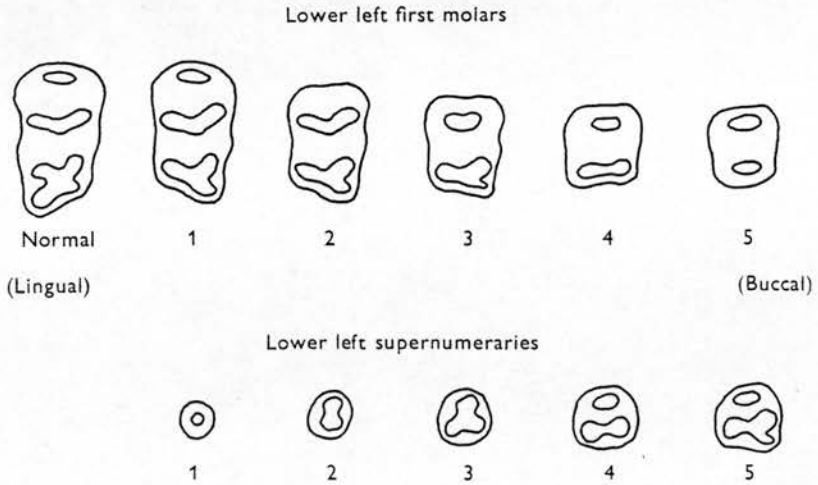


Fig. 17. Varying grades of abnormality of m_1, and varying degrees of complexity of lower supernumeraries.

Returning now to m_1, the varying grades of abnormality of this tooth are shown in Fig. 17. An interesting parallel shown in the same figure is the increase in complexity of supernumeraries with increasing size. Up to a point the complexity of supernumeraries varied as the reverse of the scale of abnormality in m_1. It therefore seems that the development of a supernumerary tooth follows a similar course to m_1 in its early stages, the degree of complexity depending on the size which is reached. However, a supernumerary was generally more complex for its size than m_1. It is also of interest that the normal m_2 is comparable with grade 1, and the normal m_3 comparable with grade 4 of m_1 abnormality. There thus appears to be a single developmental sequence common to all the lower molars. The difference between lower molars of the normal series, and between normal lower molars and lower supernumeraries, is due to different distances travelled by each tooth along a common developmental path.

2. *Variation in the incidence of lower supernumeraries*

Sofaer (1969) postulated that the development of molar supernumerary teeth in tabby mice is a response to the partial suppression of growth of the first molar of the normal series. If this is the case, the greater the suppressive effect on the developing teeth of the normal series, the greater the incidence of super-

numeraries is likely to be. A general measure of this suppressive effect is given by total tooth score. In a previous section it was shown that this expected relationship between total tooth score and the incidence of lower supernumeraries held good in heterozygotes, where they were positively correlated, but not in homozygotes and hemizygotes, where they were negatively correlated.

A model to explain these correlations is presented in Fig. 18. It has been postulated that the primary effect of the mutant genes is to suppress epithelial proliferation, but that the differentiating cells of a tooth germ are more likely to be affected by this suppression than the less well differentiated cells of the dental lamina. Using total tooth score as an indication of the level of suppression,

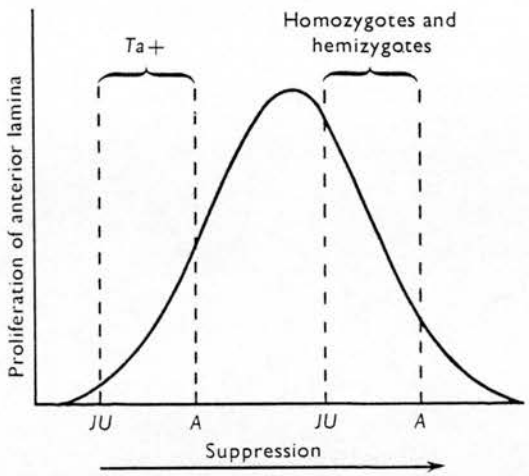


Fig. 18. A model to explain the observed incidence of lower supernumerary teeth in the different background groups.

suppression was maximum in homozygotes and hemizygotes ; within homozygotes and hemizygotes and within heterozygotes it varied with genetic background, the *A* strain background allowing maximum and the *JU* strain background allowing minimum suppression. It is proposed that as m_1 is progressively suppressed, there is an accompanying tendency for the anterior extension of dental lamina to proliferate. With further increase of the suppressive influence the dental lamina itself becomes affected, the rate of increasing incidence of proliferation becomes reduced, a maximum level is reached, and a decrease towards zero commences. It follows from Fig. 18 that the hypothetical maximum incidence of supernumerary tooth formation would occur with a level of suppression somewhere between that in the most abnormal tabby heterozygotes, and the least abnormal homozygotes and hemizygotes.

3. *Differences in local reaction to background change, and differences between the genes*

The patterns of variation of different characters within and between genes with changes of genetic background not only provide information about the relationships between the genes, but also about the level at which background modification operates.

Mimic genes are unlikely to be identical if homozygosity of any one alone is sufficient to produce the mutant phenotype. The simplest explanation is that the wild-type allele of each gene is responsible for one of a number of related steps, either in series or in parallel, towards the formation of a single end-product which is necessary for the development of a normal phenotype. Complete blockage at any point along an isolated pathway would be expected to produce an identical result. If blockage is incomplete, or if there are cross-connexions with other pathways, as seems more likely, differences between the genes may be detectable. Modification acting on the end-product of the pathway, provided it is always qualitatively the same, would cause all genes to react in the same way and all pleiotropic effects to be influenced in the same direction. Modification acting between the end-product of the pathway and the final phenotype could produce different reactions in different aspects of the phenotype within each gene. Previous studies have in fact shown that the different manifestations of a gene tend to be independently modified by genetic background (Grüneberg, 1963). If there were also differences between genes this would indicate that the nature of the end-product is not independent of the position of the genetic lesion on the pathway.

The general reaction to background change of all characters scored in the present study was in the same direction within each gene. This was shown by the maintenance of the over-all pattern of abnormality in the dentition at all levels of expression, and by the negative correlation of total vibrissa number with total tooth score and tabby heterozygote striping. However, the different groups of vibrissae, and different regions within the dentition, showed some difference in their response. The two backgrounds had opposite effects on group C and D vibrissae, and to some extent produced a different pattern of abnormality in m^1. It seems therefore that background modification has acted primarily at a fundamental level, swinging the whole pleiotropic pattern in the same direction, although there were definite, but limited, indications of secondary local interactions.

There was no evidence that the extreme phenotypes of the genes differed to any appreciable extent. At the heterozygote level, however, for every character examined, crinkled and downless tended towards complete recessivity, whereas the two tabby alleles showed variable intermediate dominance. The conclusion drawn is that the genes are qualitatively very similar, and that the quantitative differences between heterozygotes may well be solely a reflection of the difference between autosomal and sex-linkage.

SUMMARY

1. Two alleles of the sex-linked gene tabby, and two autosomal mimics of tabby—crinkled and downless—were crossed to two inbred strains which differed in their ability to favour mutant expression.

2. A consideration of the relative sensitivities to the mutant genes of different regions within the dentition suggested that the cusp pattern of a mutant molar may be basically the result of a more or less normal but rather slow course of development which has been interrupted by the relatively premature onset of calcification.

3. The incidence of lower supernumerary teeth varied widely with genetic background. In tabby heterozygotes the incidence was maximal in groups which showed the most severe general mutant effect, whereas in homozygotes and hemizygotes the incidence was maximal in the most normal animals. These results were interpreted in terms of differential sensitivity of the first molar and the dental lamina to mutant epithelial suppression.

4. Background modification caused a general swinging of the whole pleiotropic pattern in the same direction. There were, however, definite although limited signs of secondary local interactions. The genes appeared to have qualitatively very similar effects, but there was a difference in dominance in all characters examined between crinkled and downless on the one hand and the two tabby alleles on the other.

RÉSUMÉ

Aspects du syndrome 'tabby–crinkled–downless'. II.
Observations sur les réactions à des changements du fonds génique

1. Deux allèles du gène '*tabby*' lié au sexe et deux mimes autosomiques de '*tabby*', '*crinkled*' et '*downless*', ont été introduits dans deux lignées consanguines qui différaient par leur aptitude à favoriser l'expression de la mutation.

2. L'examen de la sensibilité relative aux gènes mutants de diverses régions dans la denture, a suggéré que la structure d'une molaire mutante peut être le résultat d'un développement plus ou moins normal, mais plutôt lent, qui a été interrompu par le début relativement prématuré de la calcification.

3. La présence de dents surnuméraires inférieures variait largement avec le fonds génique. Chez les hétérozygotes '*tabby*', leur fréquence était maximale dans les groupes qui présentaient l'effet général le plus grave de la mutation tandis que chez les homozygotes et les hémizygotes, la fréquence était maximale chez les animaux les plus normaux. Ces résultats ont été interprétés en termes de sensibilité différentielle de la première molaire et de la lame dentaire à l'égard de la suppression épithéliale du mutant.

4. Une modification du fonds génique a provoqué un glissement de tout le système pléiotrope dans la même direction. Il y a eu, néanmoins, des signes définis, quoique limités, d'interactions locales secondaires. Les gènes apparais-

sent avoir des effets qualitivement très semblables mais il y a eu une différence de dominance pour tous les caractères examinés, entre '*crinkled*' et '*downless*' d'une part et les deux allèles '*tabby*' de l'autre.

I am grateful to Professor D. S. Falconer for suggesting the investigation and for his interest and valuable advice during the work, to Professor C. H. Waddington for laboratory facilities, and to the Nuffield Foundation for financial support.

REFERENCES

AUERBACH, C. & FALCONER, D. S. (1949). A new mutant in the progeny of mice treated with nitrogen mustard. *Nature, Lond.* **163**, 678.

BUTLER, P. M. (1956). The ontogeny of molar pattern. *Biol. Rev.* **31**, 30–70

DUN, R. B. (1958). Growth of the mouse coat. VI. Distribution and number of vibrissae in the house mouse. *Aust. J. biol. Sci.* **11**, 95–105.

DUN, R. B. (1959). The development and growth of vibrissae in the house mouse with particular reference to the time of action of the tabby (*Ta*) and ragged (*Ra*) genes. *Aust. J. biol. Sci.* **12**, 312–20.

DUN, R. B. & FRASER, A. S. (1959). Selection for an invariant character, vibrissa number, in the house mouse. *Aust. J. biol. Sci.* **12**, 506–23.

FALCONER, D. S. (1953). Total sex-linkage in the house mouse. *Z. indukt. Abstamm.- u. VererbLehre.* **85**, 210–19.

FALCONER, D. S., FRASER, A. S. & KING, J. W. B. (1951). The genetics and development of 'crinkled' a new mutant in the house mouse. *J. Genet.* **50**, 324–44.

FRASER, A. S. & KINDRED, B. M. (1962). Selection for an invariant character, vibrissa number in the house mouse. III. Correlated responses. *Aust. J. biol. Sci.* **15**, 188–206.

FRASER, A. S., NAY, T. & KINDRED, B. M. (1959). Variation of vibrissa number in the house mouse. *Aust. J. biol. Sci.* **12**, 331–9.

GAUNT, W. A. (1955). The development of the molar pattern of the mouse (*Mus musculus*). *Acta anat.* **24**, 249–68.

GAUNT, W. A. (1956). The development of enamel and dentine on the molars of the mouse, with an account of the enamel-free areas. *Acta anat.* **28**, 111–34.

GAUNT, W. A. (1961). The development of the molar pattern of the golden hamster (*Mesocricetus auratus* W.), together with a re-assessment of the molar pattern of the mouse (*Mus musculus*). *Acta anat.* **45**, 219–51.

GRÜNEBERG, H. (1963). *The Pathology of Development.* Oxford: Blackwell.

GRÜNEBERG, H. (1965). Genes and genotypes affecting the teeth of the mouse. *J. Embryol. exp. Morph.* **14**, 137–59.

GRÜNEBERG, H. (1966). The molars of the tabby mouse, and a test of the 'single-active *X*-chromosome' hypothesis. *J. Embryol. exp. Morph.* **15**, 223–44.

KINDRED, B. M. (1967). The expression of the tabby and crinkled genes in different genetic backgrounds in the mouse. *Genetics* **55**, 173–8.

KING, J. W. B. (1956). Linkage group XIV of the house mouse. *Nature, Lond.* **178**, 1126.

LUTHER, P. G. (1949). Enzymatic maceration of skeletons. *Proc. Linn. Soc.* **161**, 146–7.

Mouse News Letter (1960). No. **23**, 30.

Mouse News Letter (1963). No. **29**, 40.

Mouse News Letter (1966*a*). No. **34**, 32.

Mouse News Letter (1966*b*). No. **35**, 24.

SOFAER, J. A. (1969). Aspects of the tabby–crinkled–downless syndrome. I. The development of tabby teeth. *J. Embryol. exp. Morph.* **22**, 181–205.

(*Manuscript received 2 December 1968*)

Archs oral Biol. Vol. 20, pp. 57 to 61. Pergamon Press 1975. Printed in Great Britain.

INTERACTION BETWEEN TOOTH GERMS AND THE ADJACENT DENTAL LAMINA IN THE MOUSE

J. A. SOFAER

University of Edinburgh, School of Dental Surgery, Edinburgh EH1 1JA, Scotland
and Department of Human Genetics, Western General Hospital, Edinburgh EH4 2HU, Scotland

Summary—In the mouse mutant tabby, an abnormal proliferation of the dental lamina sometimes develops anterior to the first molar of the normal series, apparently as a response to small size of the first molar at a critical stage of development. The abnormal proliferation may either regress or go on to form a supernumerary tooth. Measurement of adult teeth in tabby heterozygotes has disclosed three classes of first molars: small, associated with a supernumerary tooth; intermediate, presumed to have been associated with a laminal proliferation that later regressed; and large (normal). It is suggested that the discontinuities separating these three classes indicate inhibition of the developing first molar by the adjacent dental lamina at two distinct levels: a low level, produced by laminal proliferations that later regressed, and a higher level, produced by developing supernumerary teeth. It is further suggested that inhibitory interactions may be to some extent responsible for regulation during normal development, and may also have played some part in changes of tooth number during evolution.

INTRODUCTION

It has been suggested that the ability of a given region of dental lamina to proliferate and give rise to new tooth germs may be retained after the time when normal tooth germ formation in that region has been completed, the proximity of established tooth germs inhibiting a still potentially active lamina from proliferating further. This hypothesis has been put forward to explain the pattern of continuous tooth succession in the frog (Gillette, 1955) and in the lizard (Osborn, 1971), where tooth germs develop from the free margin of a persistent dental lamina beneath the spaces between the teeth of the previous tooth generation. The idea is that the point on the free margin of lamina mid-way between neighbouring established tooth germs would be the first to be released from any inhibitory influence during migration of these tooth germs towards the oral cavity and away from the free margin of lamina. Similar inhibitory influences may operate in animals where there are only one or two tooth generations, but may not be restricted to one-way effects emanating from established tooth germs and influencing the adjacent lamina. The status of the lamina itself may affect the growth of established tooth germs, and, moreover, neighbouring tooth germs may interact with one another.

An opportunity to investigate such effects presents itself in the tabby mouse. Tabby is an X-linked mutation that causes characteristic morphological changes in the molars and a general reduction of tooth size (Grüneberg, 1965). In addition, a supernumerary tooth sometimes develops anterior to the first molar of the normal series, apparently as a response to small size of the first molar at a critical stage of development (Sofaer, 1969a). A small first molar germ can be thought of as having less of an inhibitory influence on the adjacent lamina than normal, and, should the inhibitory influence fail to reach a certain threshold level, the lamina would be free to respond by forming an additional tooth. However, histological study of tabby embryos has shown that, even though the lamina may proliferate in response to a small first molar, a supernumerary tooth germ is not always formed (Sofaer, 1969a). There are, therefore, three kinds of quadrant in these mutant mice: normal quadrants, where no supernumerary laminal proliferation has occurred, quadrants where laminal proliferation has occurred but has failed to go on to form a supernumerary tooth, and quadrants containing a fully formed supernumerary tooth.

The purpose of the present paper is to discuss possible developmental consequences of abnormal laminal proliferation in tabby mice in terms of the final size of the first molar.

MATERIAL AND METHOD

Both right and left upper and lower first molars of approximately 200 adult tabby heterozygous females and approximately 200 of their normal male litter mates, already subjected to morphological analysis (Sofaer, 1969b), were measured to the nearest 1/100 mm. This was achieved with a projection microscope by projecting on a graduated screen a magnified silhouette (× 100) of each tooth to be measured. The measurement made was the maximum mesiodistal diameter of the crown parallel to the occlusal plane. For each first molar, the presence or absence of an associated supernumerary was noted. Female controls

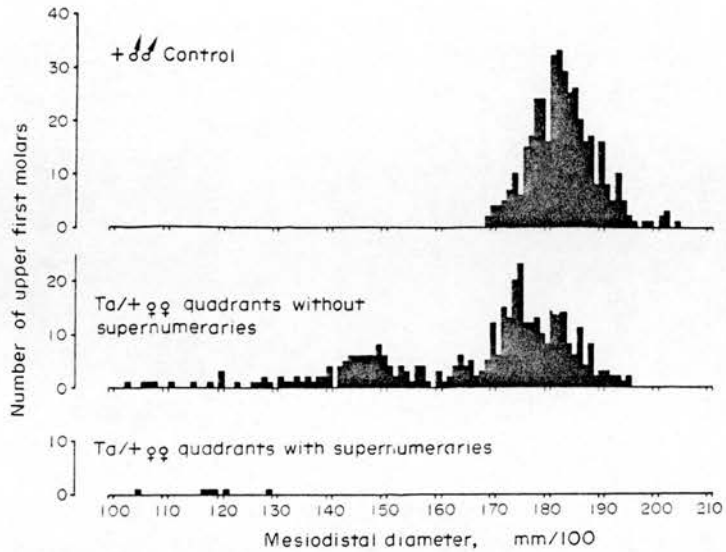


Fig. 1. The distributions of upper first molar size in normal males and their tabby heterozygote litter mates.

were not available because of the nature of the crosses that were made to produce the material, but the difference between the sexes for tooth size in the mouse appears to be very small. The proportion of the total variance of buccolingual diameter of the lower molars due to the difference between sexes has been estimated at only about 1 per cent (Bader, 1965).

RESULTS

Figures 1 and 2 show the distributions of upper and lower first molar size in normal males and their tabby heterozygote litter mates. In both upper and lower jaws, normal males form a single distribution at the top of the scale, but the distribution of heterozygotes appears to fall into three classes. First molars from quadrants with supernumerary teeth constitute a separate group at the bottom of the scale, and the distribution of first molars from quadrants without supernumerary teeth is bimodal, the upper part of this bimodal distribution corresponding to the distribution of control teeth, though, at least in the upper jaw, the sizes in this group of heterozygote teeth are, on the whole, a little smaller than normal.

DISCUSSION

Histological observation of tabby embryonic material indicates that supernumerary laminal proliferation may be associated with a smaller first molar germ than normal at the time when the abnormal proliferation first occurs. It can therefore be argued that first molar germs smaller than a certain threshold size at a critical stage of development will allow proliferation to occur, whereas those larger than the threshold size will have enough inhibitory influence to prevent the lamina from proliferating further. Assuming that the size of first molar germs at this critical stage is normally distributed, the situation can be illustrated as in Fig. 3. A possible developmental basis for the trimodal distribution of first molar size in heterozygotes is then as illustrated in Fig. 4, the intermediate teeth being assumed to have been associated with laminal proliferations that later regressed. However, it is relevant to consider why the

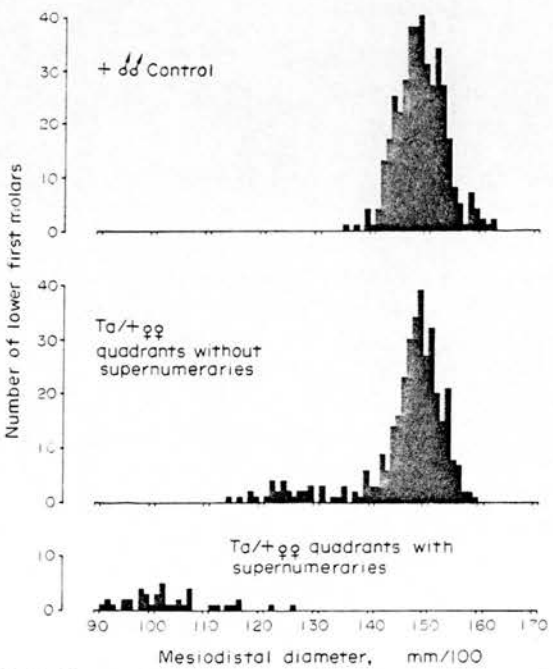


Fig. 2. The distributions of lower first molar size in normal males and their tabby heterozygote litter mates.

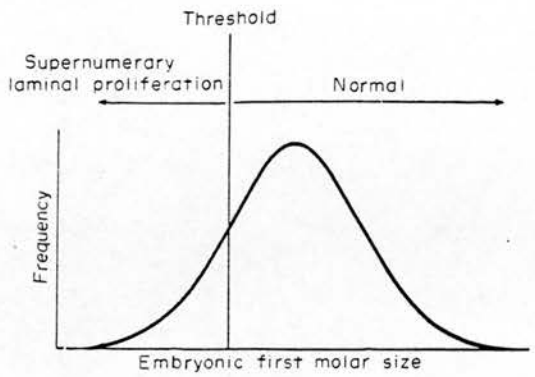


Fig. 3. The hypothetical distribution of embryonic first molar size in tabby heterozygotes. First molars below the threshold allow supernumerary laminal proliferation to occur.

three types of first molar were found to form distinct classes rather than to occupy different parts of a single continuous distribution of first molar size.

If development of the first molar is unaffected by neighbouring laminal proliferation, the distribution of adult first molar size should be similar to that in Fig. 3; that is, a single normal distribution in which first molars from quadrants where abnormal proliferation has occurred occupy the lower part, and those from normal quadrants occupy the upper part. This is illustrated in Fig. 5(a), but is not compatible with the observations. On the other hand, if, once initiated, the supernumerary laminal proliferation imposes an inhibitory influence on the first molar, the first molar would be unlikely to achieve its full growth potential. Such first molars, already small because of the mutant gene, would then be restricted further in subsequent development. In this event, first molars from quadrants in which an abnormal proliferation had occurred would not simply occupy the lower end of a single continuous distribution of adult first molar size, but would form a distinct group lower down the scale, as in Fig. 5(b). However, the distribution in Fig. 5(b) has a rather "unnatural" appearance due to the abrupt separation between its two parts. There are, in-

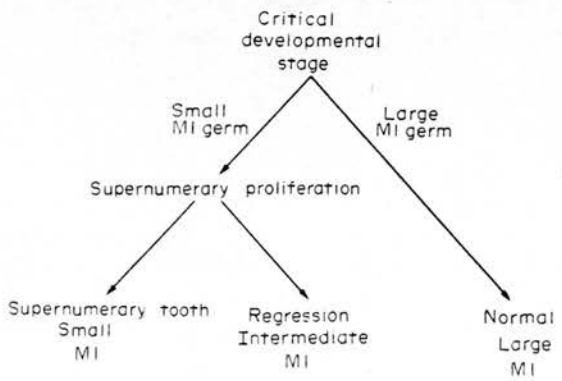


Fig. 4. Possible developmental pathways for the first molar in tabby heterozygotes.

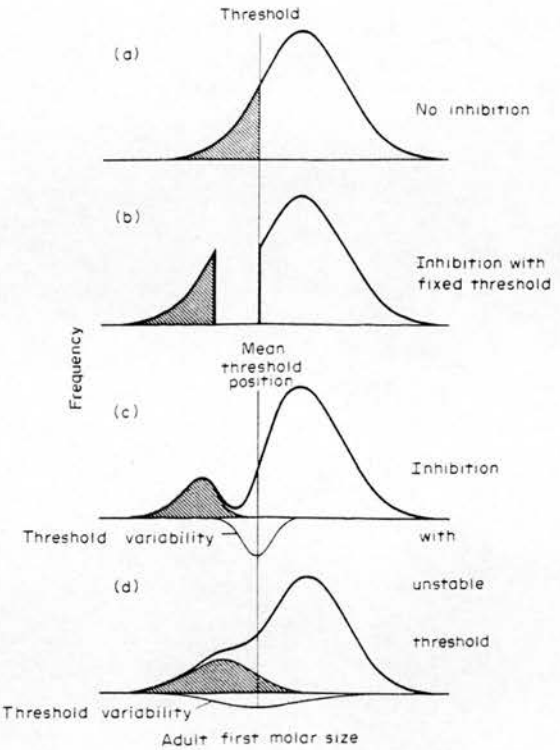


Fig. 5. Alternative hypothetical distributions of adult first molar size in tabby heterozygotes.

Note: The distributions were derived by computations based on the normal distribution, and, in each case, the hatched area indicates the distribution of first molars associated with supernumerary laminal proliferation. In (b), (c) and (d), inhibition has restricted growth of affected first molars by one standard deviation of the complete distribution shown in (a). In (c), the standard deviation of the threshold is 1/3 the degree of restriction (the distance between the two halves of the distribution in (b), and in (d) it is equal to the degree of restriction.

deed, reasons why separation should not be so precise. Firstly, the position of the threshold at the critical stage of development may be variable. This means that in some cases a first molar of a particular size would allow a supernumerary proliferation to occur, whereas in others it would not. Secondly, the size of the first molar at the critical stage might not be a clear indication of its size in the adult animal. If growth rates varied among first molars after the critical stage, the final distribution of adult first molar size would be affected in a way similar to that resulting from variation in the position of the threshold. As these two possible effects cannot be separated, they are considered together here as "threshold instability". Figures 5(c) and 5(d) illustrate the final distributions of adult first molar size with different degrees of threshold instability, instability being represented in each case by an inverted normal curve showing the variability of threshold position. The kind of distribution in Figs. 5(c) and 5(d) does seem to be compatible with the observations.

The results can therefore be accounted for in terms of inhibition of the developing first molar by adjacent laminal activity, but instead of two classes of first molar appearing there are three: small first molars associated with supernumerary teeth (presumably exposed to a long period of inhibition), intermediate first molars presumed to have been associated with supernumerary laminal proliferations that later regressed (and therefore exposed to a short period of inhibition) and large first molars presumed to have developed normally. The separation between the classes appears to be in the order of that shown in Fig. 5(c), implying that the differences of growth restriction between the classes due to different levels of inhibition could have been in the region of three times the standard deviation of each threshold (see explanation of Fig. 5).

A criticism that might be made of this interpretation concerns the mosaicism shown by heterozygotes for X-linked genes in mammals and the single active X-chromosome hypothesis (Lyon, 1972). It could be argued that the different classes of first molar developed from tooth germs in which the majority of cells contained either the mutant or the normal allele on the active X-chromosome, the predominantly mutant germs allowing laminal proliferation to occur and the predominantly normal germs developing normally. However, an additional mechanism would have to be invoked to account for the third category of adult teeth. Furthermore, the non-random involvement of the dentition in these mutants (Grüneberg, 1966) and evidence for a diffusible gene product (McLaren, Gauld and Bowman, 1973) suggest that mosaicism may not be manifested on the morphological level in tabby heterozygotes. The trimodal distribution of first molar size in tabby heterozygotes therefore seems likely to be neither due to, nor incompatible with, X-chromosome inactivation.

The discontinuous nature of the distribution of adult first molar size disclosed here suggests that, once supernumerary laminal proliferation has occurred, whether or not the proliferation goes on to form a supernumerary tooth, the growth of the adjacent first molar is inhibited. Thus the proliferation of the relatively undifferentiated cells of the lamina, as well as the presence of established supernumerary germs, may affect the developing first molar. However, the inhibitory influence could be mutual. The intermediate cases are possibly ones in which an overwhelming inhibitory influence from the first molar, perhaps because it is only just below the size threshold, prevents laminal proliferation from progressing further. Whether the inhibitory effect is a specific one or whether it arises because of competition for common growth requirements is not clear. In either event, the interaction between germ and germ, and between germ and lamina, can provide a developmental mechanism whereby the length of the tooth row tends to be stabilized.

The implication for ontogeny is that size regulation of developing tooth germs can work towards providing a quantity of tooth material appropriate to the local conditions. It has already been shown that, in cases of unilateral absence of the upper lateral incisors in man, the central incisor on the side where the lateral incisor is missing tends to be larger than on the side where the lateral incisor is present and of normal size (Sofaer, Chung, Niswander and Runck, 1971). From the phylogenetic point of view, it is evident that the mechanisms discussed here could be responsible for changes of tooth number during evolution. If some genetic influence arises (either a major genetic factor like tabby or a combination of minor factors) and the inhibitory influence of established tooth germs relative to the potential activity of the lamina is thereby reduced, an additional tooth may appear. Conversely, if genes occur that increase the inhibitory influence of teeth that develop early, later developing teeth that had previously been normal components of the dentition may be eliminated.

The present example has shown how a mutant gene can disrupt an apparently stable pattern of dental development and uncover relationships that may be difficult to detect under normal conditions. The relationships disclosed indicate that tooth size may be regulated to some extent by local interactions during development, and that genetic changes causing alteration in the balance of these interactions may be responsible for changes of tooth number during evolution.

Acknowledgements—I am grateful to Professor J. M. Thoday, University of Cambridge, Department of Genetics, for laboratory facilities, and to Christa Lucas for making the tooth measurements. Financial support was in the form of a Nuffield Foundation Dental Research Fellowship and a Research Project Grant from the Medical Research Council.

REFERENCES

Bader R. S. 1965. A partition of variance in dental traits of the house mouse. *J. Mammal.* **46**, 384–388.

Gillette R. 1955. The dynamics of continuous succession of the teeth in the frog (*Rana pipiens*). *Am. J. Anat.* **96**, 1–36.

Grüneberg H. 1965. Genes and genotypes affecting the teeth of the mouse. *J. Embryol. exp. Morph.* **14**, 137–159.

Grüneberg H. 1966. The molars of the tabby mouse, and a test of the 'single active X-chromosome' hypothesis. *J. Embryol. exp. Morph.* **15**, 223–244.

Lyon M. F. 1972. X-chromosome inactivation and developmental patterns in mammals. *Biol. Rev.* **47**, 1–36.

McLaren A., Gauld I. K. and Bowman P. 1973. Comparison between mice chimaeric and heterozygous for the X-linked gene *tabby*. *Nature, Lond.* **241**, 180–183.

Osborn J. W. 1971. The ontogeny of tooth succession in *Lacerta vivipara* Jacquin (1787). *Proc. R. Soc.* B, **179**, 261–289.

Sofaer J. A. 1969a. Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *J. Embryol. exp. Morph.* **22**, 181–205.

Sofaer J. A. 1969b. Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. *J. Embryol. exp. Morph.* **22,** 207–227.

Sofaer J. A., Chung C. S., Niswander J. D. and Runck D. W. 1971. Developmental interaction, size and agenesis among permanent maxillary incisors. *Hum. Biol.* **43,** 36–45.

Archs oral Biol. Vol. 22, pp. 299 to 301. Pergamon Press 1977. Printed in Great Britain.

SHORT COMMUNICATION

THE TEETH OF THE "SLEEK" MOUSE

J. A. SOFAER

University of Edinburgh, School of Dental Surgery, Edinburgh EH1 1JA
and Department of Human Genetics, Western General Hospital,
Edinburgh EH4 2HU, Scotland

Summary—The autosomal dominant mutant "sleek" (*Slk*) in the mouse produces the same syndrome of dental abnormalities as is known to occur in homozygotes and hemizygotes for the X-linked gene "tabby" (*Ta*), and in homozygotes for the autosomal recessive genes "crinkled" (*cr*, chromosome 13) and "downless" (*dl*, chromosome 10). Incisors and third molars are small or absent. First and second molars are usually smaller than normal and have a characteristic morphology. A supernumerary molar germ sometimes develops anterior to the first molar of the normal series, either in the upper jaw or the lower jaw, and may either develop into a separate tooth, or become fused to the first molar to form a composite double tooth.

The autosomal dominant mutant "sleek" (*Slk*) in the mouse recently arose spontaneously in a stock maintained at the MRC Radiobiology Unit, Harwell (Cattanach, 1975). The general phenotypic effects of *Slk* appear to be indistinguishable from those found in homozygotes and hemizygotes for the X-linked gene "tabby" (*Ta*), and in homozygotes for the autosomal recessive genes "crinkled" (*cr*, chromosome 13) and "downless" (*dl*, chromosome 10). An examination of the dentitions of 25 sleek heterozygotes (*Slk*/+) confirmed that the sleek gene produces the same syndrome of dental abnormalities as is already known to occur in tabby, crinkled or downless mice.

The normal mouse dentition is composed of one continuously growing incisor and three molars of limited growth in each quadrant. The upper molars are referred to as m^1, m^2 and m^3, the lower molars as m_1, m_2 and m_3, and their surfaces as anterior, posterior, buccal and lingual. The crown of m^1 has eight cusps, all of which are tilted posteriorly. Numbered from anterior to posterior there are three central cusps, 1, 2 and 3; three buccal, B1, B2 and B3; and two lingual, L1 and L2. All the cusps present in m^1, except cusp 1, are represented in m^2. The crown of m_1 has seven cusps, most of which are tilted anteriorly. Numbered from anterior to posterior there are three buccal, B1, B2 and B3, three lingual, L1, L2 and L3, and a single central posterior cusp, 4. All cusps present in m_1, except L1 and sometimes B1, are represented in m_2. The cusp patterns of the third molars are simpler and subject to some variation. Figure 1a and d show the crowns of normal upper and lower molars. Both m^1 and m^2 have three roots, anterior and posterior buccal roots and a single lingual root. Both m_1 and m_2 have two roots, one anterior and one posterior. Third molars usually have single roots that may show a tendency towards division into three (m^3) or two (m_3) at a variable distance from the apex.

All the dental abnormalities found in sleek mice have previously been reported for tabby, crinkled and downless (Grüneberg, 1965; Sofaer, 1969a and b). In sleek mice, the incisors may be reduced or absent. The crown of m^1 is smaller, more bulbous and has more erect cusps than normal. Cusp B1 of m^1 is always absent, and cusp B3 of m^1 is usually absent. Cusps L1 and L2 of m^1 are not as distinctly separated from each other and from cusp 1 as they are in the normal mouse, and, in some cases, complete absence of separation between these cusps produces a single cusp lingually that is continuous with cusp 1. The root of m^1 is usually single, though there are sometimes two or even three roots. The crown of m^2 is also more bulbous than normal, and cusp B3 is usually absent. Cusp B1 of m^2 may be of normal size, but is sometimes larger than normal. There is always a ridge of variable height, connecting cusps B1 and L1, running transversely across the anterior end of the crown of m^2. Grüneberg (1965) has called this a "rampart". Cusps L1 and L2 of m^2 are often smaller than normal. The whole crown of m^2 may be small or of about normal size, but is sometimes larger than normal, probably as a compensatory reaction to the small size of m^1. Usually, m^2 has a single root, but sometimes there may be two and rarely three roots. The crown of m_1 is reduced anteriorly and posteriorly. Cusps L1 and 4 of m_1 are always absent, and cusp B1 is often absent. The separation between cusps B3 and L3, and between cusps B2 and L2 of m_1, is always less distinct than normal and, in extreme cases, each of these pairs of cusps may be represented by a single cusp only. There are usually two roots, but sometimes only one. In some cases, m_1 is smaller than m_2. Nevertheless, the crown of m_2 is also always reduced anteriorly and posteriorly. Cusps B1 and 4 of m_2 are always absent. Cusps B3 and L3 of m_2 are poorly separated and may be represented by a single cusp, and cusps B2 and L2

may be affected similarly. There may be one or two roots. Upper and lower third molars are either small or absent. Mutant upper and lower molars, showing the most common situation found in sleek heterozygotes, are illustrated in Fig. 1b and e.

Other abnormalities have been found occasionally in the dentitions of tabby, crinkled and downless mice: a supernumerary incisor, either separate from or fused to the adjacent incisor of the normal series; a supernumerary molar anterior to the first molar of the normal series, either with the third molar present, resulting in a quadrant containing four molars, or with the third molar absent; fusion between the first molar and an adjacent supernumerary, producing a composite double molar tooth. Instances of all these abnormalities, except supernumerary incisors, were found in the *Slk*/+ material examined (Fig. 1c, f, g).

The situation in tabby heterozygotes is somewhat different and a little more complicated (Grüneberg, 1965, 1966; Sofaer, 1969a and b, 1975).

The severity of the abnormalities produced by tabby, crinkled and downless can be influenced by background genotype. A similar background effect could probably be demonstrated for sleek by making appropriate crosses to different inbred strains. Minor differences in severity of effect between sleek mice and tabby, crinkled or downless mice may not therefore be due to differences between the mutant genes themselves, but rather to interaction with other genes that happen to be present in the individual. However, a rudimentary cusp B3 was found in m^1 and m^2 of six out of the 25 sleek heterozygotes examined, whereas no cusp B3 has been detected in the upper molars of a total of approximately 500 tabby hemizygotes and tabby, crinkled and downless homozygotes on a variety of genetic backgrounds (Sofaer, 1969b).

There are therefore four mutant genes in the mouse, each at its own locus, and each of which is alone capable of producing the same syndrome of dental abnormalities. The simplest explanation is that the wild-type allele of each gene is responsible for one of a number of related steps, either in series or in parallel, towards the formation of a single end product that is necessary for normal development. These steps may be either at the biochemical level, or at levels of cell or tissue interactions. If it becomes possible to study the genes at these more fundamental levels, differences between them will probably become apparent. There is already some evidence to suggest that slightly different abnormalities of epithelium–mesenchyme interaction are involved in tabby and downless mice (Sofaer, 1974).

This report may be of interest to those working with, or contemplating work with, the dentitions of tabby, crinkled or downless mice. Sleek could be used either as an additional source of material or as an alternative, because an autosomal dominant gene may have advantages over X-linked and autosomal recessive genes from the points of view of stock maintenance and experimental design.

Acknowledgement—I am grateful to Dr. Bruce Cattanach for the gift of sleek mice.

REFERENCES

Cattanach B. M. 1975. Private communication. *Mouse News Lett.* **53**, 29.

Grüneberg H. 1965. Genes and genotypes affecting the teeth of the mouse. *J. Embryol. exp. Morph.* **14**, 137–159.

Grüneberg H. 1966. The molars of the tabby mouse, and a test of the 'single-active X-chromosome' hypothesis. *J. Embryol. exp. Morph.* **15**, 223–244.

Sofaer J. A. 1969a. Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *J. Embryol. exp. Morph.* **22**, 181–205.

Sofaer J. A. 1969b. Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. *J. Embryol. exp. Morph.* **22**, 207–227.

Sofaer J. A. 1974. Differences between *tabby* and *downless* mouse epidermis and dermis in culture. *Genet. Res., Camb.* **23**, 219–225.

Sofaer J. A. 1975. Interaction between tooth germs and the adjacent dental lamina in the mouse. *Archs oral Biol.* **20**, 57–61.

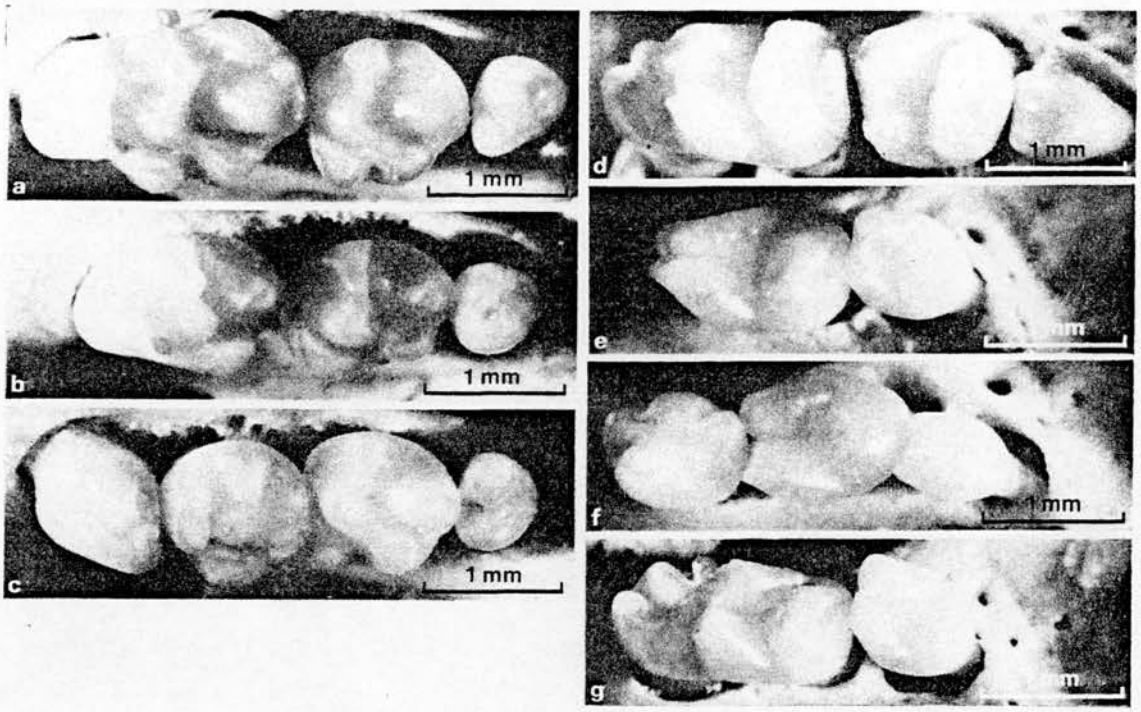


Fig. 1. Bucco-occlusal views of upper left molars (a, b, c) and linguo-occlusal views of lower right molars (d, e, f, g) from normal (+/+) and sleek (*Slk*/+) mice. Starting from the left, the teeth shown are: (a) m^1, m^2, m^3 (+/+); (b) m^1, m^2, m^3 (*Slk*/+); (c) Supernumerary, m^1, m^2, m^3 (*Slk*/+); (d) m_1, m_2, m_3 (+/+); (e) m_1, m_2 (m_3 absent, *Slk*/+); (f) Supernumerary, m_1, m_2 (m_3 absent, *Slk*/+); (g) Composite tooth (fused supernumerary and m_1), m_2 (m_3 absent, *Slk*/+). Note: The explanations given for c, f and g are based on what is known of the dental embryology of tabby mice.

J. Embryol. exp. Morph. Vol. 41, pp. 279–287, 1977
Printed in Great Britain © *Company of Biologists Limited 1977*

Tooth development in the 'crooked' mouse

By J. A. SOFAER[1]

From the University of Edinburgh,
School of Dental Surgery and the
Department of Human Genetics,
Western General Hospital, Edinburgh

SUMMARY

The semidominant gene 'crooked' (*Cd*) in the mouse produces anomalies of the axial skeleton (resulting in a crooked tail), microphthalmia and dental abnormalities, including small molars with simplified cusp patterns that are equivalent to patterns passed through during normal morphodifferentiation. A series of embryonic litters from $Cd/+ \times Cd/+$ matings was used to investigate the embryological basis for the dental abnormalities. Microphthalmic embryos were classed as *Cd/Cd*, and their most normal litter mates were selected as controls ($+/+$ or $Cd/+$). An additional set of control embryos came from the inbred strain CBA/Cam ($+/+$). Serial sagittal sections of the heads of these embryos were examined microscopically, and the maximum anteroposterior diameters of the developing upper and lower first molars were measured. Reduction in the rates of growth and morphodifferentiation of *Cd/Cd* first molars, relative to those of litter mate controls, was associated with the appearance of an adjacent abnormal proliferation of the dental lamina. Some proliferations in older embryos showed signs of early tooth germ formation, but many were seen to have regressed and no examples of supernumerary teeth have been found in *Cd/Cd* adults. Small size of *Cd/Cd* molars may therefore result from competitive inhibition of molar growth by a transient abnormal laminal proliferation, and *Cd/Cd* cusp patterns from the relatively premature onset of hard tissue formation during normal but retarded sequences of morphodifferentiation.

INTRODUCTION

The semidominant autosomal gene 'crooked' in the mouse (also known as 'crooked-tail', symbol *Cd*) produces a variety of malformations when homozygous. Among these are anomalies of the axial skeleton, a relatively elongated head with a pointed snout and abnormal ear inclination, microphthalmia, a tail that has irregular tail rings and is sparsely populated with abnormal hairs, and dental abnormalities. Homozygosity is frequently lethal, either before or soon after birth. In the original mutant stock only an estimated 28 % of homozygotes survived the perinatal period, and the majority of these were infertile. Of the 72 % of homozygotes that did not survive beyond birth, about one third were thought to have suffered lethality before implantation. In heterozygotes, the abnormalities of homozygotes are expressed to a much lesser degree, malformations often being confined to the lumbo-sacral and caudal regions of the

[1] *Author's address:* School of Dental Surgery, Chambers Street, Edinburgh EH1 1JA, U.K.

vertebral column. In the original mutant stock about 11 % of heterozygotes were phenotypically normal, and all appeared to be normally viable and fertile. As a result of the anomalies of the caudal skeleton surviving homozygotes and some heterozygotes have sharp bends in the tail. The effects of the gene were first described by Morgan (1954) and have been further discussed by Grüneberg (1963).

Dental abnormalities of crooked mice have previously been reported by Grewal (1962) and Grüneberg (1965), but before describing these it is appropriate to review the main features and nomenclature of normal mouse teeth. The normal mouse dentition is composed of one continuously growing incisor and three molars of limited growth in each quadrant of the jaws. The upper molars are referred to as m^1, m^2 and m^3, the lower molars as m_1, m_2 and m_3, and their surfaces as anterior, posterior, buccal and lingual. The crown of m^1 has eight cusps, all of which are tilted posteriorly. Numbered from anterior to posterior there are three central cusps, 1, 2 and 3; three buccal, B1, B2and B3; and two lingual, L1 and L2. All the cusps present in m^1, except cusp 1, are represented in m^2. The crown of m_1 has seven cusps, most of which are tilted anteriorly. Numbered from anterior to posterior there are three buccal, B1, B2 and B3; three lingual, L1, L2 and L3; and a single central posterior cusp, 4. All the cusps present in m_1, except L1 and sometimes B1, are represented in m_2. The cusp patterns of the third molars are simpler and subject to some variation. Both m^1 and m^2 have three roots, anterior and posterior buccal roots and a single lingual root. Both m_1 and m_2 have two roots, one anterior and one posterior. Third molars usually have single roots that may show a tendency towards division into three (m^3) or two (m_3) at a variable distance from the apex.

In *Cd*/*Cd* mice the lower incisors are either small or absent. The upper incisors are of about normal size, but, if not worn down by an opposing lower, grow round in a spiral. The crowns of the first and second molars, particularly the uppers, are smaller and more bulbous than normal. In m^1, cusp 1 is relatively large and more erect than normal, cusp B1 is absent, and cusps L1 and L2 are not as distinctly separated from each other and from cusp 1 as they are in the normal mouse. The anterior and lingual roots are usually fused. In m^2, all members of the normal complement of cusps are usually represented, but all are reduced in size and less distinctly separated from each other than normal. This lack of distinctness is most severe on the lingual side, where there may be an undivided large cusp in the place of cusps L1 and L2. The cusp patterns of the lower first and second molars are less abnormal than those of the uppers. In m_1, cusps B1 and L1 are poorly separated and cusp 4 is relatively small. In m_2, cusp B1 may be reduced or absent, and cusp 4 is usually absent. Bifurcation of the roots of both m_1 and m_2 usually occurs a little further towards the root apices than normal. Upper, and particularly lower third molars, are either relatively small or altogether absent. Excellent illustrations of normal and *Cd*/*Cd* teeth are given by Grüneberg (1965). The dental morphology of *Cd*/+ mice is usually

Table 1. *The numbers of embryos of each genotype sectioned at each stage, followed in brackets by the numbers of litters from which they were taken. Left sides only of CBA mice, but both sides of mice from the mutant stock were sectioned*

Stage	CBA/Cam	Litter mate controls	*Cd/Cd*
Day 14	10 (3)	2 (2)	2 (2)
15	10 (3)	2 (2)	3 (2)
16	10 (3)	2 (2)	3 (2)
17	10 (3)	2 (2)	3 (2)
18	10 (3)	2 (2)	4 (2)

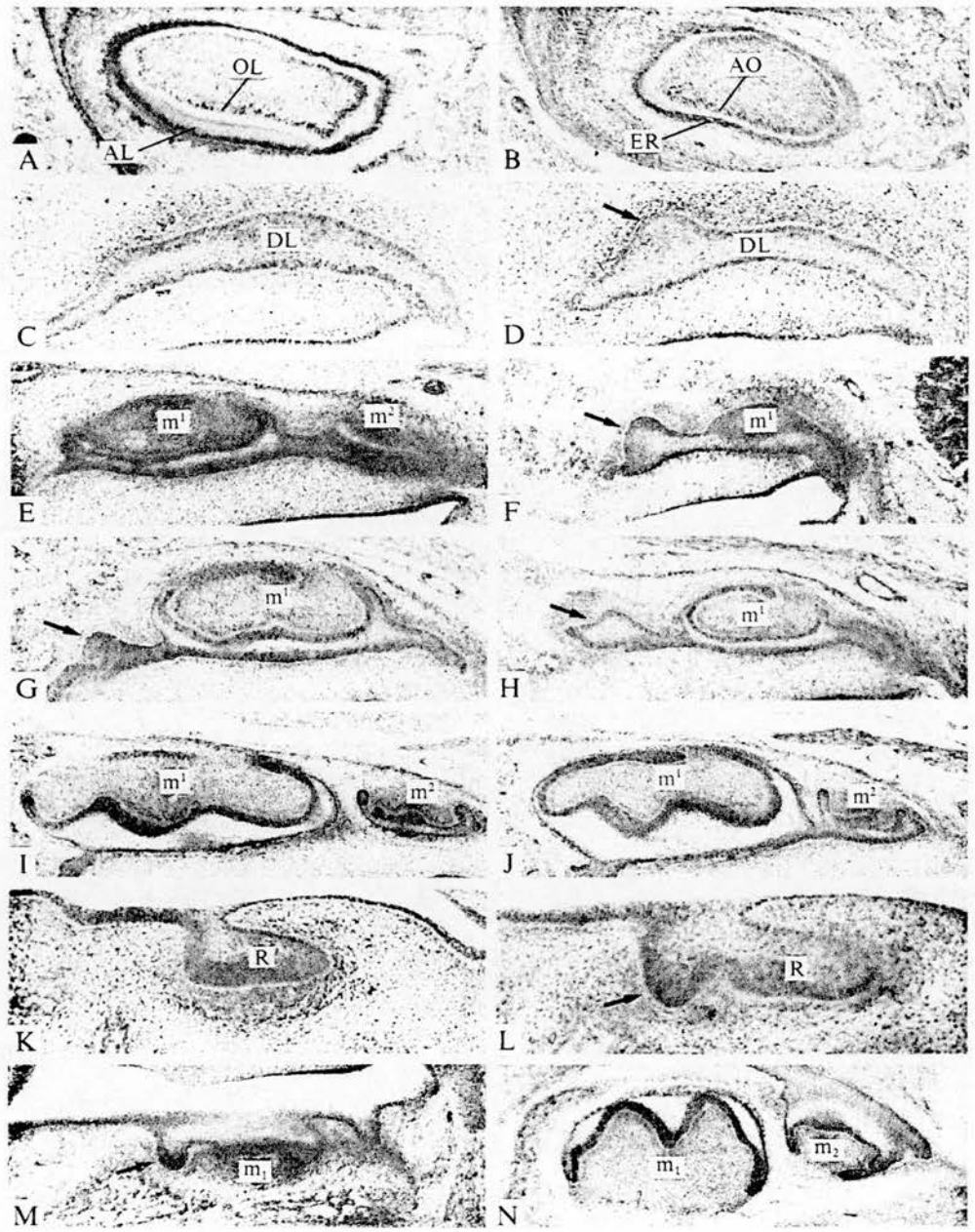
normal, though the teeth may be slightly smaller than those of wild-type mice from the same stock (Grewal, 1962).

The present paper is concerned with the embryology of the dental abnormalities in crooked homozygotes, particularly those of the molar crowns. This is an aspect of *Cd/Cd* mice that has not previously been investigated.

MATERIAL AND METHOD

Embryos from litters of $Cd/+ \times Cd/+$ matings were obtained from two sources: University College London; and the author's own mutant stock, founded by University College animals. The gestational stage was determined either by an examination of the external features of the embryos (Grüneberg, 1943), or by a combination of timing from the day on which a vaginal plug was found (day zero) and an examination of external features of the resulting litter. Litters of days 14, 15, 16, 17 and 18 were used. Embryos with microphthalmia were classed as *Cd/Cd*, and the most normal and well-developed member of each litter was selected as a litter-mate control (presumed $+/+$ or $Cd/+$). In addition, a series of embryos from the inbred strain CBA/Cam, wild-type at the crooked locus, was used for comparison. The heads of all embryos were fixed in Bouin's fluid, serially sectioned at 10 μm in the sagittal plane and stained with haematoxylin and eosin. The numbers of embryos sectioned at each stage are shown in Table 1.

The serial sections were examined, and, for *Cd/Cd* mice and their litter mate controls, the maximum anteroposterior diameter of each tooth germ, from the earliest stage at which it could be measured (day 14 for m_1 and day 15 for m^1), was taken from images produced by a projection microscope at a standard magnification.



0·5 mm I, J, N

0·5 mm A, B, E, F, G, H, M

0·5 mm C, D, K, L

RESULTS

No clear difference was found between litter mate control embryos and CBA/Cam embryos at any of the stages examined. Dental development in *Cd/Cd* embryos can therefore be compared with both groups of controls as a whole.

No difference was observed between the upper incisor germs of *Cd/Cd* mice and those of their controls. By contrast, the lower incisor germs of *Cd/Cd* mice showed definite differences from the controls, particularly from day 16 onwards, and a considerable degree of variation. Some *Cd/Cd* lower incisor germs were fairly normal in size and histodifferentiation for their developmental stage, whereas others were smaller, with abnormal odontoblasts and no ameloblasts (Fig. 1 A, B).

A difference between the upper molar regions of *Cd/Cd* mice and controls was observed at day 14. In one out of four *Cd/Cd* upper quadrants (two embryos) examined, there was a small knot of cells arising from the anterior half of the band of dental lamina that later would have given rise to the upper first molar germ. This knot, which appeared to have been produced by a localized proliferation of laminal cells, was not present in control embryos (Fig. 1 C). Larger and more definite laminal proliferations were found in five out of six *Cd/Cd* upper quadrants at day 15 (Fig. 1 D), and larger proliferations still in all of the six *Cd/Cd* upper quadrants at day 16 (Fig. 1 E and F). At day 17, the proliferation was either absent or appeared to be regressing (four out of six *Cd/Cd* upper quadrants, Fig. 1 G), or had progressed further to an early stage of tooth germ formation with an associated mesenchymal condensation (two out of six *Cd/Cd* upper quadrants, Fig. 1 H). In the two cases of early tooth germ formation found at day 17 the first molar germs were smaller than those in the four cases where the proliferation had either regressed or was absent. At day 18, the proliferation was either reduced or absent in all of the eight *Cd/Cd* upper quadrants examined. The more bulbous shape of *Cd/Cd* upper molar germs, compared with controls, was clearly visible on days 17 and 18 (Fig. 1 I and J).

Fig. 1. 10 μm sagittal sections through *Cd/Cd* and control (CBA/Cam) embryos at different gestational ages. Anterior to the left. AL, ameloblast layer; OL, odontoblast layer; ER, remnants of internal enamel epithelium; AO, abnormal odontoblasts; DL, dental lamina; R, rudiment of m_1. Arrows indicate abnormal proliferations of the dental lamina. See text under 'Results' for full explanation. (A) Control upper incisor, day 18. (B) *Cd/Cd* upper incisor, day 18. (C) Control upper molar region, day 14. (D) *Cd/Cd* upper molar region, day 15. (E) Control upper molar region, day 16. (F) *Cd/Cd* upper molar region, day 16. (G) *Cd/Cd* upper molar region, day 17. (H) *Cd/Cd* upper molar region, day 17. (I) Control upper molars, day 18. (J) *Cd/Cd* upper molars, day 18. (K) Control lower molar region, day 14. (L) *Cd/Cd* lower molar region, day 15. (M) *Cd/Cd* lower molar region, day 16. (N) Control lower molars, day 18.

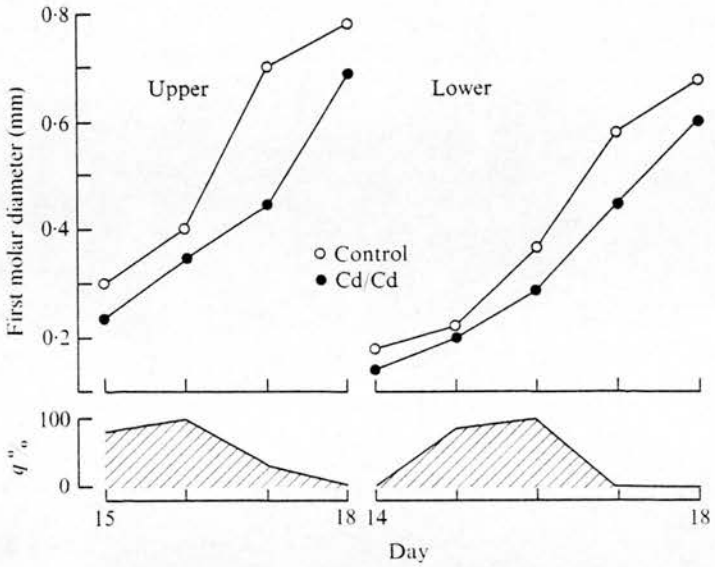


Fig. 2. Mean anteroposterior diameters of first molar germs for *Cd/Cd* embryos and their litter mate controls at different gestational ages, related to the proportion of jaw quadrants containing 'active' laminal proliferations (q %).

No difference between the lower molars of *Cd/Cd* mice and those of controls was observed at day 14 (Fig. 1 K), but at day 15 there was evidence of an abnormal proliferation of the dental lamina anterior to the point of origin of the first molar germ in five out of six *Cd/Cd* lower quadrants (Fig. 1 L). At day 16, all six *Cd/Cd* lower quadrants showed signs of abnormal laminal proliferation (Fig. 1 M), but at days 17 and 18 the abnormal outgrowth was either much reduced or absent in all *Cd/Cd* lower quadrants, and *Cd/Cd* lower molar germs were morphologically indistinguishable from those of controls (Fig. 1 N).

Measurement of the anteroposterior diameters of upper and lower first molar germs showed that the mean diameter of *Cd/Cd* germs was always less than the corresponding mean diameter for litter mate controls. The difference in size between *Cd/Cd* germs and their controls appeared to be related to the proportion of *Cd/Cd* quadrants in which 'active' laminal proliferations were observed; that is, definite proliferations that were larger than those of the previous stage and where there were no signs of regression. The difference in size was greatest at day 17, one day after the maximum incidence of active proliferations in both upper and lower jaws (Fig. 2).

DISCUSSION

The most general manifestation of the crooked gene in the dentition is small size of the fully formed teeth. The measurements of developing first molar germs, summarized in Fig. 2, show that after an initial period when tooth germ size was similar in *Cd*/*Cd* embryos and their controls, there was a divergence between the rates of growth of mutant and control first molars. This divergence was associated with the appearance of an abnormal proliferation of the dental lamina in both the upper and lower jaws. Figure 2 suggests that the relative growth rates of mutant and control first molars are not maintained, but that, after initial divergence, the growth of *Cd*/*Cd* first molar germs tends to run parallel to that of their controls. Equivalent tooth size in the two genotype groups is separated by a time lag of approximately one day, though the lag is greater in the upper jaw than in the lower.

All parts of m^1 and m_1 affected by the gene are those appearing late in the ontogeny of normal first molar crowns, and m^1 is more severely affected than m_1 (Grüneberg, 1965). The different time lags suggested by Fig. 2 may provide an explanation for this. If hard tissue formation occurs at the same chronological age in both *Cd*/*Cd* and normal embryos, irrespective of molar size and morphodifferentiation (and there was no indication to the contrary in the present material), the resulting morphology of fully formed mutant first molars would be as is found, namely, corresponding to an incomplete level of normal morphodifferentiation with the upper first molar more incomplete than the lower. The small size and simplified morphology of *Cd*/*Cd* first molars might therefore be explained, at least in part, by competitive inhibition of first molar growth due to an adjacent proliferation of the dental lamina. It has already been mentioned that within the present mutant material first molar size was smallest in quadrants where the laminal proliferations were most advanced. There is good evidence that interaction between an abnormal laminal proliferation and first molar growth also occurs in heterozygotes for the *X*-linked gene 'tabby' (*Ta*) in the mouse (Sofaer, 1975).

However, competitive inhibition of first molar growth is unlikely to be the whole explanation, because *Cd*/*Cd* second molars are also smaller than normal, and third molars are frequently absent. Nevertheless, it is possible that the effect of abnormal laminal proliferation may persist for second and third molars, even though a proliferation itself is no longer present. It appears, both from observation and experiment, that all three molars of one quadrant arise from cells that, at early stages of tooth germ formation, are represented by the first molar germ and its immediately associated epithelium; that is, the three molars of one quadrant do not have clearly independent origins from different points along the dental lamina (Lumsden & Osborn, 1976). More convincing evidence in favour of an additional, perhaps more fundamental, cause of the dental abnormalities comes from the lower incisor findings. These imply that

there is an intrinsic deficiency in the internal enamel epithelium, or its interaction with the adjacent preodontoblasts, that prevents it from differentiating into ameloblasts (Fig. 1A, B). This observation also provides another interesting parallel with tabby, where homozygotes and hemizygotes show an abnormality of incisor histodifferentiation indistinguishable from that illustrated in Fig. 1B (Sofaer, 1969*a*).

There are further points of similarity between the dental abnormalities of *Cd*/*Cd* mice and homozygotes (or hemizygotes) for tabby, its recessive autosomal mimics 'crinkled' (*cr*) and 'downless' (*dl*) (Grüneberg, 1965; Sofaer, 1969*b*), and its more recently discovered dominant autosomal mimic 'sleek' (*Slk*) (Cattanach, 1975; Sofaer, 1977). The first molar crowns are all smaller and more bulbous than normal and have simplified cusp patterns. The missing cusps in both the upper and the lower molars are those that develop late in the normal mouse, with one exception. Cusp B3 of m^1 and m^2, which appears early in the ontogeny of normal upper molar crowns, and which is present in *Cd*/*Cd* mice, is always missing in tabby, crinkled and downless. However, in sleek, which in every other respect is indistinguishable from tabby, crinkled and downless, cusp B3 has been found in about one quarter of the dentitions examined.

A point of difference between *Cd*/*Cd* mice and tabby, crinkled, downless and sleek occurs in the upper second molars. These have always been found to be smaller than normal in *Cd*/*Cd* mice, whereas they are sometimes larger than normal in tabby and its mimics. A possible explanation for this is that the effect of tabby and its mimics seems to be a timed one, causing suppression of tooth germ growth during particular phases of development. Early growth of the first molar occurs during such a suppression phase, whereas early growth of the second molar occurs at a time when suppression has been relaxed, and when there is therefore an opportunity for compensatory increase in size (Sofaer, 1969*a*). The absence of cusp B3 could also be due to this suppression phase, in that the cusp normally appears first during the time when suppression would be operative. In the sleek mice examined, cusp B3 may have developed because of a slight shift or shortening of the suppression phase.

One of the most interesting features of tabby, crinkled, downless and sleek dentitions is the occasional occurrence of a supernumerary tooth anterior to the first molar of the normal series in both upper and lower jaws. These teeth develop from a proportion of the abnormal laminal proliferations, the remainder regressing before a tooth germ becomes established. In a large sample of tabby heterozygotes, probably the majority of abnormal proliferations in the upper jaw, and about half of those in the lower jaw, regressed without forming a supernumerary tooth (Sofaer, 1975). It has been shown here that early stages of tooth germ formation do occur in *Cd*/*Cd* mice. It therefore seems likely that a proportion of the abnormal proliferations of *Cd*/*Cd* mice also go on to form supernumerary teeth, and that if a larger number of *Cd*/*Cd* adults were examined such teeth might be found.

The *Cd*/*Cd* dentition therefore has some features in common with the dentitions of tabby, crinkled, downless and sleek mice. The skeletal abnormalities of crooked mice, the majority of which have been shown to be a consequence of irregular somite formation (Grüneberg, 1963), suggest that there is a defect in the mesenchymal element of developing *Cd*/*Cd* dentitions. By contrast, experiments with downless (Sofaer, 1973) and crinkled (Mayer, Miller and Green, 1977) indicate that, in these mutants, it is the epidermal component that is at fault. Similar experiments with tabby have produced equivocal results (Sofaer, 1974), and sleek has not been investigated in this way. Nevertheless, the evidence available suggests that a reduction in tooth-germ growth and the formation of bulbous tooth germs, associated with abnormal proliferations of the dental lamina and even failure of histodifferentiation of the internal enamel epithelium, can be produced by primary defects in either the epithelial or the mesenchymal component of a developing dentition.

The author is grateful to Professor H. Grüneberg for his encouragement to undertake the study, to Professor Grüneberg and Dr G. M. Truslove for the gift of *Cd*/+ mice and fixed embryonic litters, and to Miss Edith Redpath for technical assistance.

REFERENCES

CATTANACH, B. M. (1975). Private communication. *Mouse News Letter* **53**, 29.

GREWAL, M. S. (1962). The development of an inherited tooth defect in the mouse. *J. Embryol. exp. Morph.* **10**, 202–211.

GRÜNEBERG, H. (1943). The development of some external features in mouse embryos. *J. Hered.* **34**, 88–92.

GRÜNEBERG, H. (1963). *The Pathology of Development.* Oxford: Blackwell Scientific Publications.

GRÜNEBERG, H. (1965). Genes and genotypes affecting the teeth of the mouse. *J. Embryol. exp. Morph.* **14**, 137–159.

LUMSDEN, A. & OSBORN, J. W. (1976). Development of the mouse dentition in culture. *J. dent. Res.* **55**, Special issue D, p. D136 (Abstract).

MAYER, T. C., MILLER, C. K. & GREEN, M. C. (1977). Site of action of the crinkled (*cr*) locus in the mouse. *Devl. Biol.* **55**, 397–401.

MORGAN, W. C. (1954). A new crooked tail mutation involving distinctive pleiotropism. *J. Genet.* **52**, 354–373.

SOFAER, J. A. (1969*a*). Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *J. Embryol. exp. Morph.* **22**, 181–205.

SOFAER, J. A. (1969*b*). Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. *J. Embryol. exp. Morph.* **22**, 207–227.

SOFAER, J. A. (1973). Hair follicle initiation in reciprocal recombinations of *downless* homozygote and heterozygote mouse tail epidermis and dermis. *Devl. Biol.* **34**, 289–296.

SOFAER, J. A. (1974). Differences between *tabby* and *downless* mouse epidermis and dermis in culture. *Genet. Res., Camb.* **23**, 219–225.

SOFAER, J. A. (1975). Interaction between tooth germs and the adjacent dental lamina in the mouse. *Archs oral Biol.* **20**, 57–61.

SOFAER, J. A. (1977). The teeth of the 'sleek' mouse. *Archs oral Biol.* **22**, 299–301.

(*Received 25 March 1977, revised 10 May 1977*)

J. Embryol. exp. Morph. Vol. 26, 1, pp. 99–109, 1971
Printed in Great Britain

The genetics and development of fused and supernumerary molars in the rice rat

By J. A. SOFAER[1] AND J. H. SHAW[2]
From the National Institutes of Health, Bethesda, and the Harvard School of Dental Medicine

SUMMARY

Analysis of breeding records suggests that the occurrence of fused and supernumerary molars in the rice rat, associated with lower body weight than normal and with reduced fertility, is dependent on a single autosomal recessive gene subject to background modification.

Molar fusion, which is preceded by stripping of the external enamel epithelium from the interdental lamina, may involve the first two molars, or all three molars of the normal series in either jaw.

The supernumerary is a posterior tooth, developing after the three molars of the normal series in either jaw. Supernumerary development usually occurs with fusion of the molars of the normal series, but occasionally a supernumerary may be present in the absence of fusion.

Other examples of association between fusion, subsequent to separation of the external enamel epithelium from the interdental lamina, and supernumerary tooth development are cited. This association suggests that there may be a single common attribute of the dental lamina predisposing to epithelial stripping and to laminal hyperactivity.

INTRODUCTION

The occurrence of fused and supernumerary molars among members of a colony of rice rats (*Oryzomys palustris*), and the relationship between this trait and body weight, have been reported briefly elsewhere (Griffiths & Shaw, 1961; Shaw, Griffiths & Osterholtz, 1963). Originally, a few affected individuals were found sporadically with what appeared to be fusion of the first and second molars; and in some animals a supernumerary tooth was present, apparently posterior to the third molar. Subsequently, selection for animals with abnormal teeth rapidly resulted in a strain with a high incidence of fused and supernumerary molars. The fused and supernumerary molar trait was associated with lower body weight than normal, and breeding experience indicates that fertility in the fused molar strain is reduced. In other respects fused and supernumerary molar animals are outwardly normal.

Comparable but not identical abnormalities are found in the mouse mutants

[1] *Author's address:* Department of Genetics, Milton Road, Cambridge CB4 1XH, U.K.
[2] *Author's address:* Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, Massachusetts 02115, U.S.A.

tabby (*Ta*), crinkled (*cr*) and downless (*dl*) (Grüneberg, 1965, 1966; Sofaer, 1969), and by a selected line of Lakeland Terriers (Hitchin & Morris, 1966). In the mutant mice, supernumerary teeth sometimes develop anterior to the first molar, and occasionally fusion between the supernumerary and first molar occurs. In the dog, fusion of the deciduous or permanent incisors has been observed, with or without a related supernumerary tooth.

The present paper is concerned with an analysis of the genetics of the condition in the rice rat; and with a consideration of the development of the abnormal dentition based on observations in adult animals and on direct examination of embryological material. The relationship of the abnormal dental condition in the rice rat to those in the mouse and dog is also discussed.

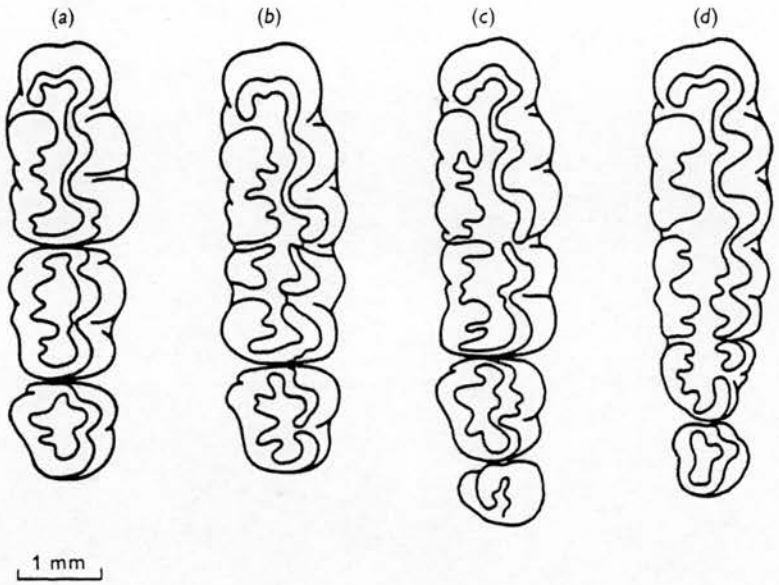


Fig. 1. Diagrams of occlusal views of rice rat upper right molars: (*a*) normal; (*b*)–(*d*) from the fused molar and supernumerary molar strain.

MATERIALS AND METHODS

The normal rice rat dentition is composed of one incisor and three molars in each quadrant. Fig. 1(*a*) illustrates the occlusal view of the three upper right molars in a normal animal. In abnormal animals molar fusion may occur with or without a supernumerary being present, and conversely, but infrequently, a supernumerary may be present without molar fusion.

Examination of several hundred abnormal animals suggests that the supernumerary tooth is nearly always situated posterior to the third molar and is the last tooth to develop. Fig. 1(*b*) shows a case where fusion of the first and second molars has apparently taken place. In such cases the composite tooth is nearly always slightly shorter than the combined length of the normal first and second

molars, but some compensation often seems to have occurred through enlargement of the presumed third molar. Fig. 1(*c*) shows a similar case with an additional tooth situated posteriorly. In this kind of case the combined length of the composite tooth and presumed third molar is usually less than in case (*b*) where no supernumerary is present. Fig. 1(*d*) shows a case where all three molars of the normal series appear to have fused, and there is an additional tooth situated posteriorly. Similar cases without the supernumerary tooth have been observed frequently. Very occasionally (about 1 % of the time) the additional tooth is not situated posterior to the third molar but lingual to, and in contact with, the second and third molars. Similar situations occur in the lower jaw.

It should be mentioned here that the composite teeth shown in illustrations (*b*)–(*d*) of Fig. 1 describe the maximum degree of maintenance of normal size and morphology of the component molars that was observed. In many cases reduction in size was greater, and in some cases, where reduction was extreme, it was not possible to identify all cusps of the component molars in the composite tooth.

For the genetic analysis, the distributions of offspring produced by mating phenotypically different combinations of animals were studied from the mating records kept over the 5-year period from 1963 to 1967 at the Harvard School of Dental Medicine. Each animal was classified according to the number of quadrants affected by either fusion or a supernumerary, or both. Throughout these discussions an 'affected' animal is one in whom 1–4 quadrants were affected. The affected and non-affected conditions are abbreviated as *A* and *NA* respectively.

The embryological material consisted of 23 animals, from both the fused strain and a normal control strain, ranging in age from an estimated 4–5 days before birth to 5 days after birth. These 23 animals were serially sectioned at 8 μm in the sagittal plane and processed by routine histological procedures.

RESULTS AND DISCUSSION

1. *Genetics*

Table 1 shows the distribution of progeny of different kinds of mating according to the number of quadrants affected. The distribution clearly shows that the condition is inherited, but simple single gene inheritance could not apply since $A \times A$ matings produced some *NA* progeny and since $NA \times NA$ matings also produced some *A* progeny. Further examination of $A \times A$, $A \times NA$ and $NA \times NA$ matings, subdivided by sex, provided no evidence of any sex-linked effect. The two possible extreme hypotheses that could account for the inheritance of the condition are therefore (1) the segregation of two alleles at a single autosomal locus, with incomplete penetrance in abnormal homozygotes, and (2) multifactorial inheritance.

Multifactorial control would classify the condition as a quasi-continuous

character, dependent on some underlying continuous scale but expressed only above a certain threshold. A feature of such characters is the positive relationship between the proportion of affected individuals in a given population and the mean degree of expression of the character among those individuals who are affected (Grüneberg, 1952). Table 2 shows rank correlations between the percentage of *A* and the mean number of quadrants involved among *A* progeny, for different kinds of mating. The matings are divided into two groups, one in which at least one parent was *NA*, and the other in which both parents were *A*.

Table 1. *The number of quadrants affected and the percentage of affected individuals among progeny of three different kinds of mating*

		No. of progeny						
		Quadrants affected						
Mating	No. of matings	0	1	2	3	4	Total	*A* (%)
$A \times A$	185	113	101	453	304	1209	2180	94·8
$A \times NA$	24	213	28	64	27	43	375	43·2
$NA \times NA$	54	1050	15	37	13	31	1146	8·4

There is no correlation in the first group, but a very high and significant correlation in the second. This suggests that the underlying variation among progeny of $A \times A$ matings is continuous, but that this continuity does not extend below the threshold when *NA* parents are involved. The implication is, then, that there is a relatively discrete difference between *A* individuals and at least some *NA* individuals. The fact that matings in which at least one parent was *NA*, particularly the 0×0 matings, produced *A* progeny with a relatively high mean number of quadrants affected suggests that there may have been segregation of a major recessive factor contributing to the abnormal dental condition.

The left half of Fig. 2 illustrates distributions of families of different kinds of mating according to the percentage of affected individuals they contained, each family comprising the total progeny (ranging from 2 to 47) of a single pair. There is overwhelmingly only one kind of family among $A \times A$ matings, but possibly three among $A \times NA$ matings, and probably more than one among $NA \times NA$ matings. This pattern is compatible with the hypothesis of a single autosomal recessive gene with incomplete penetrance in homozygotes, as shown below.

Under the single autosomal recessive hypothesis all *A* individuals must be considered homozygous, say *ff*, whereas *NA* individuals could have any one of the three genotypes: $++$, $f+$ or *ff*. There would then be only one kind of $A \times A$ mating, producing somewhat less than 100 % *A* offspring ($ff \times ff$); three kinds of $A \times NA$ mating, producing no *A* offspring ($ff \times ++$), somewhat less than 50 % *A* ($ff \times f+$), and somewhat less than 100 % *A* ($ff \times ff$); and four kinds of $NA \times NA$ mating, producing no *A* offspring ($++ \times ++$; $++ \times f+$, $++ \times ff$), somewhat

less than 25 % A ($f+ \times f+$), somewhat less than 50 % A ($f+ \times ff$), and somewhat less than 100 % A ($ff \times ff$).

Having obtained this pattern of family distributions the mating records were re-examined to try to establish the genotypes, according to the single autosomal recessive hypothesis, of NA parents of $A \times NA$ matings, and of both parents of those $NA \times NA$ matings that produced some A offspring. Non-affected individuals whose parents were both affected were considered homozygous mutant

Table 2. *Rank correlations between the percentage of affected progeny and the mean number of quadrants involved among affected progeny, for matings in which at least one parent was non-affected, and for matings in which both parents were affected*

Matings by quadrants affected	No. of progeny	A (%)	Mean no. of quadrants involved among A progeny	Rank correlations between % A and mean of quadrants involved
0 × 0	1146	8	2·6	
0 × 1	11	18	1·5	
0 × 2	83	43	2·0	Spearman = −0·10, Kendall = 0
0 × 3	51	61	2·4	
0 × 4	232	39	2·8	
1 × 1	83	70	1·7	
1 × 2	58	71	2·0	
1 × 3	22	55	1·6	
1 × 4	41	90	2·4	Spearman = 0·95 ($P < 0·01$).
2 × 2	262	92	2·3	Kendall = 0·87 ($P < 0·01$)
2 × 3	91	96	2·5	
2 × 4	146	95	2·9	
3 × 3	83	95	3·1	
3 × 4	263	97	3·3	
4 × 4	1131	99	3·8	

(ff), those whose ancestors were consistently NA over several generations were considered homozygous normal ($+ +$), and those with one presumed ff parent and one presumed $+ +$ parent were considered heterozygous ($f+$). The results of this re-examination are shown in Table 3. The 'probable' heterozygotes were offspring of parents who were both presumed to be heterozygous themselves.

The right half of Fig. 2 illustrates distributions of progeny of the three different kinds of $A \times NA$ mating, and of the three different kinds of $NA \times NA$ mating that could produce affected offspring. Of the $A \times NA$ matings, none classified as $ff \times + +$ produced any A offspring. (Of the two $A \times NA$ families that produced between 1 and 20 % A progeny, shown in the left half of Fig. 2, one was classified as $ff \times f+$ and the other as $ff \times ff$.) The distribution of offspring of $A \times NA$ matings classified as $ff \times f+$ strongly suggests segregation into a non-affected group and a group with a relatively high degree of expressivity, with rather less than half of the total affected. Offspring of $A \times NA$ matings

classified as $ff \times ff$ showed no evidence of any such bimodality, and had a mean degree of effect somewhat lower than the presumed ff homozygous progeny of the $ff \times f+$ matings. This difference of expressivity between the two groups of presumed homozygotes could be accounted for by a difference of genetic background, since the homozygous parent of the group with high expressivity was always affected, whereas one homozygous parent of the group with low expressivity was always non-affected.

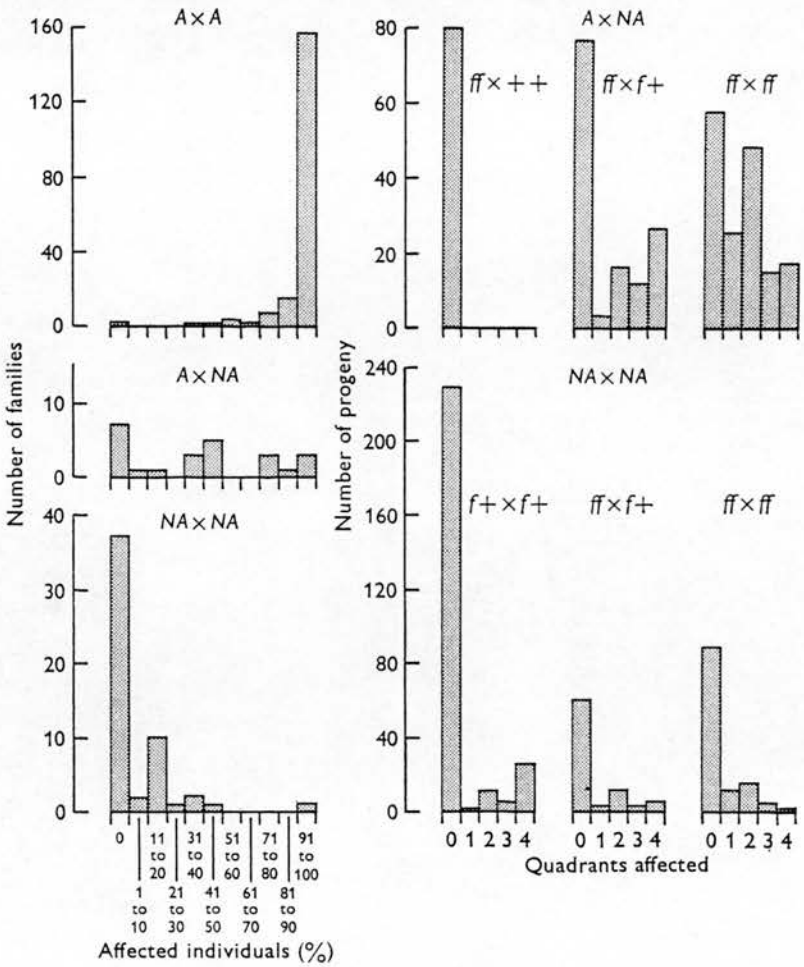


Fig. 2. Distribution of families of phenotypically different kinds of mating according to the percentage of affected individuals the families contained (left); and distributions of progeny of genotypically different kinds of mating, based on the single autosomal recessive hypothesis, according to the number of quadrants affected (right).

The distribution of progeny of $NA \times NA$ matings classified as $f+ \times f+$ was again strongly suggestive of segregation, with rather less than one quarter of the total affected, and with relatively high expressivity among the affected progeny. Evidence for segregation among the progeny of $NA \times NA$ matings classified as

ff×*f*+ was not so convincing, possibly due again to a background effect imposed by a non-affected homozygous *ff* parent. The progeny of *NA* × *NA* matings classified as *ff*×*ff* showed no evidence of bimodality, and expressivity was rather lower than among offspring of *A* × *NA* matings classified as *ff*×*ff*. This also could be due to background, since in the group with lower expressivity both *ff* parents were non-affected.

Table 3. *Presumed genotypes of non-affected parents, derived from an examination of their ancestry, according to the single autosomal recessive hypothesis*

	NA parent of *A* × *NA* matings			*NA* × *NA* matings that produced some *A* offspring		
	++	*f*+	*ff*	*f*+ × *f*+	*ff*×*f*+	*ff*×*ff*
Definite	6	4	10	6	0	6
Probable	0	4	0	2	3	0

The existence of a heritable background effect among presumed *ff* homozygotes was tested for by calculating the heritability of the number of quadrants affected from the regression of the mean of offspring on mid-parent value for the 10 different *A* × *A* matings listed in Table 2. The result, $h^2 = 0{\cdot}94$, indicates that a very high proportion of the variation of expression among presumed *ff* homozygotes was due to additive genetic effects.

Thus, the observations are all compatible with the hypothesis of a single autosomal recessive gene with variable penetrance in homozygotes, with penetrance being largely dependent on genetic background.

It should be mentioned here that the apparently rather low numbers of individuals in the 1-quadrant and 3-quadrant classes of the distributions in the right half of Fig. 2 are probably due to relative developmental instability of the asymmetrical condition, since when two quadrants were affected they were most frequently in the same jaw. The relative stability of the 1-, 2- and 3-quadrants affected conditions can in fact be calculated if certain assumptions are made. The first requirement is a basically homogeneous group of individuals which can be taken as being normally distributed on some underlying continuous scale immediately related to the development of the phenotype. Different ranges on this underlying scale are assumed to correspond to different grades of phenotype (each grade being a different number of quadrants affected), and these different ranges are assumed to be separated by constant thresholds. The relative sizes of the intervals between the thresholds can then be derived from the frequencies of the different grades of phenotype (Falconer, 1964, 1965; Rendel, 1967). Calculation of the relative sizes of the 1-, 2- and 3-quadrant threshold intervals from the distribution of progeny of all *A* × *A* matings, on the assumption of normality of this distribution, showed that the 2-quadrant interval was twice as large as either the 1- or the 3-quadrant interval.

2. *Development*

At approximately 4 days before birth early morphodifferentiation of first and second molars, and the rudiment of the third molar were observed in both normal and fused molar animals (Fig. 3A, B). In fused molar animals the external enamel epithelium between adjacent tooth germs was generally well separated from the underlying dental lamina, resulting in continuity of the stellate reticulum of all three developing teeth (Fig. 3B). Normal animals showed a slight tendency towards separation (Fig. 3A), but in no case was it observed to be as extreme as in the most abnormal fused molar animal.

By one day after birth hard tissue formation had begun in both first and second molars (Fig. 3C), and in fused molar animals fusion had been completed (Fig. 3E, G). Cases were observed in which fusion of the first and second molars had taken place (Fig. 3E), and in these cases the third molar germ was developmentally more advanced than normal. In normal animals invagination of the third molar germ was only just beginning, whereas in the abnormal animals a well-defined bell had already been formed (Fig. 3E). Other cases were observed with a large composite germ in which there was a greater antero-posterior range of histodifferentiation than that seen in fused first and second molars. The antero-posterior length of these composite teeth (Fig. 3G) was similar to the sum of the lengths of the fused first and second molar and advanced third molar of the type illustrated in Fig. 3E. It was concluded that these composite teeth had developed from the rudiments of all three molars of the normal series. In one such case, the rudiment of an additional tooth was observed posteriorly (Fig. 3G, inset).

At 5 days after birth normal third molars had reached the late bell stage (Fig. 3D). In abnormal animals in which the third molar had remained separate, differentiation of the third molar was again more advanced than normal, and in some cases an extension of dental lamina distal to the third molar showed signs of early odontogenic activity (Fig. 3F). Previous work (Grewal, 1962; Sofaer, 1969) suggests that such supernumerary buds may or may not be destined to form additional teeth. In the one abnormal animal of this age examined in which all three molars of the normal series had apparently fused, there was an additional germ, distal to the composite tooth, which had reached the early bell stage (Fig. 3H).

Lower jaw findings were similar.

The mechanism of molar fusion in the rice rat seems to be basically the same as that proposed by Hitchin & Morris (1966) to account for fusion of the developing incisors of the dog. Following stripping of the external enamel epithelium from the interdental lamina, which was thought to be due to rapid growth of adjacent tooth germs, the internal enamel epithelia of adjacent germs would be free to come into contact and to fuse. The reason for epithelial stripping in the rice rat is not clear. However, once separation between the external enamel epithelium and the interdental lamina has occurred, it is easy

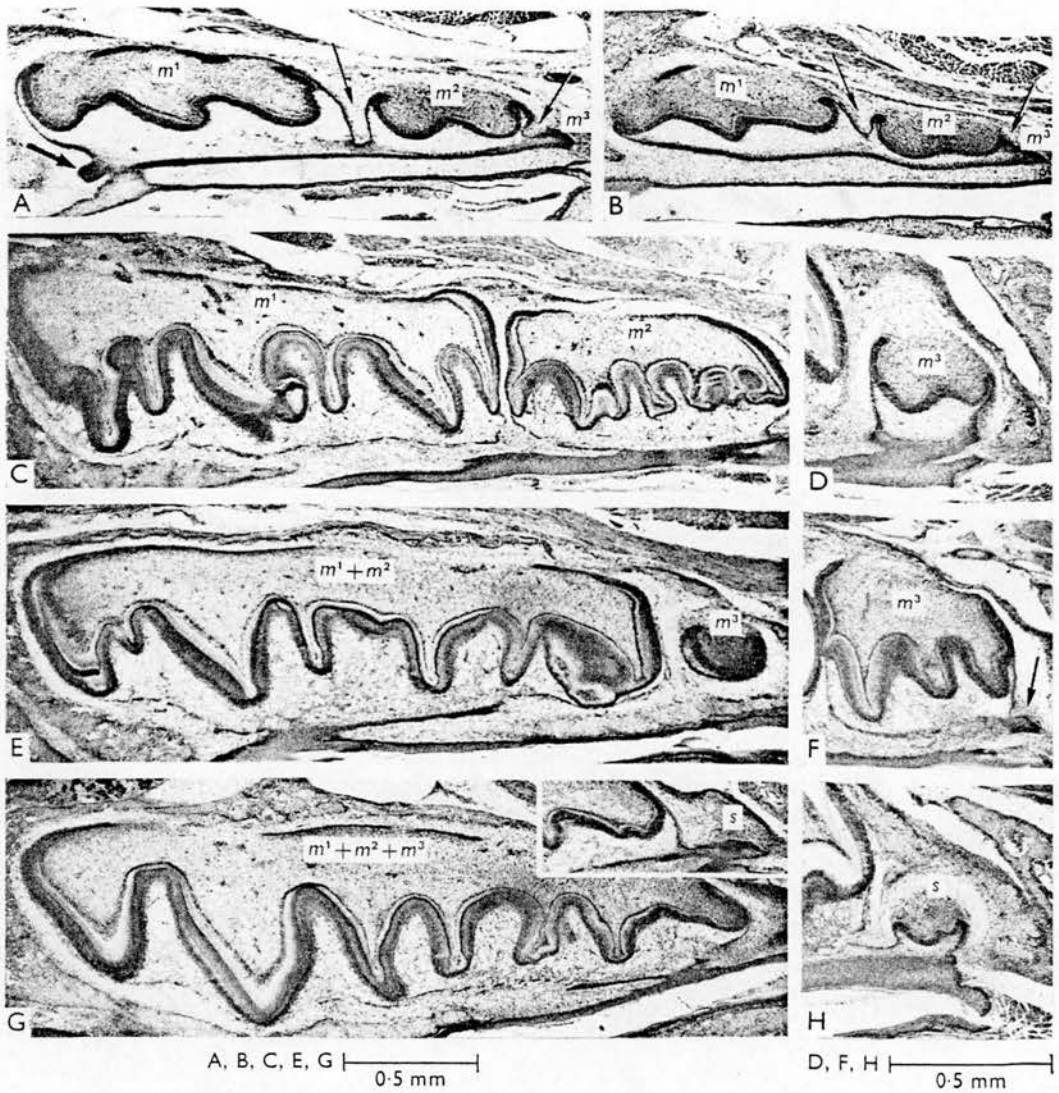


Fig. 3. m^1, m^2, m^3, upper first, second and third molars; *s*, supernumerary.
(A) Control first and second molars, and the rudiment of the third molar, at an estimated 3–4 days before birth. Light arrows indicate points of relationship between the external enamel epithelium and underlying dental lamina. Heavy arrow indicates the normal anterior extension of dental lamina.
(B) First and second molars, and the rudiment of the third molar, at an estimated 4–5 days before birth, in an animal from the abnormal strain. Light arrows indicate points of separation of the external enamel epithelium from the underlying dental lamina.
(C) Control first and second molars at one day after birth.
(D) Control third molar at 5 days after birth.
(E) Fused first and second molars and developmentally advanced third molar, of an animal from the abnormal strain at one day after birth.
(F) Developmentally advanced third molar, and rudiment of a potential supernumerary tooth (indicated by arrow), of an animal from the abnormal strain at 5 days after birth.
(G) Fused first, second and third molars, and the rudiment of a supernumerary tooth (inset), of the same animal from the abnormal strain at one day after birth.
(H) Supernumerary tooth germ from a case as in (G) but at 5 days after birth.

to understand how the case illustrated in Fig. 3B could develop into that illustrated in Fig. 3G.

Despite the similarities between the rice rat and the tabby mouse, the origin of the supernumerary molar in the two animals is not the same. In the tabby mouse the supernumerary develops anterior to but later than the first molar, resulting in adult cases in which the most anterior molar (the supernumerary) is smaller than its neighbour. In no animal of the several hundred abnormal adult rice rats examined was the most anterior molar smaller than the tooth immediately posterior to it. Furthermore, histological examination failed to reveal evidence of proliferation of the normal anterior extension of dental lamina (illustrated in Fig. 3A). In addition, there was direct evidence for the development of an additional tooth both posterior to a third molar that had remained separate (Fig. 3F), and posterior to a composite tooth thought to be derived from rudiments of all three molars of the normal series (Fig. 3G, H). Therefore, as suspected from an examination of adult material, the rice rat supernumerary appears to be a posterior tooth.

A further difference between the rice rat and the tabby mouse concerns the relationship between fusion and the supernumerary. In the tabby mouse, fusion was always found to be secondary to supernumerary formation, and fusion always involved the supernumerary. As already mentioned, fusion may occur in the rice rat without a supernumerary being present, and conversely, a supernumerary may be present in the absence of fusion. Similarly, in the dog, fusion was not found to be dependent on the presence of a supernumerary tooth.

In the tabby mouse it was suggested that the supernumerary represented an attempt to compensate for small size of the developing first molar, caused by a suppressive influence at a particular stage of development. Subsequent relaxation of the suppressive influence was thought to result in rapid growth of the supernumerary and adjacent first molar in a restricted space, predisposing to stripping of the external enamel epithelium from the interdental lamina, and to fusion. In the rice rat, epithelial stripping apparently occurs without undue crowding of the developing teeth (Fig. 3B), and the supernumerary develops only after fusion has taken place. The supernumerary tooth in the rice rat may arise in some cases in response to a smaller than normal combined antero-posterior length of the molars of the normal series, consequent to fusion. On the other hand, since supernumeraries occasionally occur in the absence of fusion, this could not be the only explanation for their development.

In conclusion then, it seemed reasonable to suspect that the different kinds of association between fusion and the development of supernumerary teeth discussed here reflect a single common attribute of the dental lamina predisposing to epithelial stripping and to laminal hyperactivity.

Support for the rice rat colony was provided in part by U.S.P.H.S. research grant D-1355 from the National Institute of Dental Research, National Institutes of Health.

REFERENCES

FALCONER, D. S. (1964). *Introduction to Quantitative Genetics.* Edinburgh: Oliver and Boyd.

FALCONER, D. S. (1965). The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Ann. hum. Genet., Lond.* **29**, 51–76.

GREWAL, M. S. (1962). The development of an inherited tooth defect in the mouse. *J. Embryol. exp. Morph.* **10**, 202–211.

GRIFFITHS, D. & SHAW, J. H. (1961). Fused molars and supernumerary molars in the rice rat. *J. dent. Res.* **40**, 731–732.

GRÜNEBERG, H. (1952). Genetical studies on the skeleton of the mouse. IV. Quasi-continuous variations. *J. Genet.* **51**, 95–114.

GRÜNEBERG, H. (1965). Genes and genotypes affecting the teeth of the mouse. *J. Embryol. exp. Morph.* **14**, 137–159.

GRÜNEBERG, H. (1966). The molars of the tabby mouse, and a test of the 'single-active *X*-chromosome' hypothesis. *J. Embryol. exp. Morph.* **15**, 223–244.

HITCHIN, A. D. & MORRIS, I. (1966). Geminated odontome–connation of the incisors in the dog – its etiology and ontogeny. *J. dent. Res.* **45**, 575–583.

RENDEL, J. M. (1967). *Canalisation and Gene Control.* London: Academic Press.

SHAW, J. H., GRIFFITHS, D. & OSTERHOLTZ, M. (1963). Relationship between body weight and occurrence of the fused molar and supernumerary molar traits in the rice rat. *Archs oral Biol.* **8**, 777–778.

SOFAER, J. A. (1969). Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *J. Embryol. exp. Morph.* **22**, 181–205. II. Observations on the reaction to changes of genetic background. *J. Embryol. exp. Morph.* **22**, 207–227.

(*Received 25 November 1970*)

Archs oral Biol. Vol. 27, pp. 701 to 703, 1982
Printed in Great Britain.

0003-9969/82/080701-03$03.00/0

SHORT COMMUNICATION

NATURALLY-OCCURRING EXPOSURE OF THE DENTAL PULP IN MICE WITH INHERITED HYPOPHOSPHATAEMIA

J. A. SOFAER*† and J. C. SOUTHAM*

*Department of Oral Medicine and Oral Pathology, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR and
†University Department of Human Genetics, Western General Hospital, Edinburgh EH4 2XU, Scotland, U.K.

Summary—The X-linked mouse mutant hypophosphataemia (*Hyp*) is a homologue of human hypophosphataemia (vitamin-D-resistant rickets). In addition to dental abnormalities already reported, exposure of the dental pulp occurs frequently through developmental deficiency of the dentine.

Deficiencies of the dentine are a feature of human X-linked hypophosphataemia (vitamin-D-resistant rickets). The deficiencies typically take the form of elongated pulp horns that stretch towards, and sometimes reach, the dentine–enamel junction. These dentine abnormalities may be associated with microscopic cracks or deficiencies in the overlying enamel, resulting in channels of access for microorganisms from the mouth to the pulp chamber. As a consequence, in human hypophosphataemia there may be multiple periapical abscesses in the absence of any obvious clinical dental pathology (Marks, Lindahl and Bowden, 1965; Archard and Witkop, 1966; Bixler, 1976).

The X-linked mouse mutant hypophosphataemia (*Hyp*, Eicher *et al.*, 1976) provides an animal model that appears to be entirely homologous with the human condition, both human and mouse disorders being caused by a primary renal defect (Bulfield, 1981). The dental abnormalities shown by a small number of these mice were reported by Iorio *et al.* (1979a,b), but without reference to exposure of the dental pulp. Our purpose is simply to draw attention to the fact that naturally occurring exposure of the dental pulp may be found in hypophosphataemic mice, just as in the human condition.

Hypophosphataemic mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, U.S.A. (*C57BL/6J-Hyp*) and the stock was maintained by two types of mating: (1) *Hyp*/+ females × +/*Y* males, from which normal (+/*Y*) and mutant (*Hyp*/*Y*) male offspring of different ages were studied; (2) +/+ females × *Hyp*/*Y* males, from which all female offspring (*Hyp*/+) were studied. Hypophosphataemic males were easily distinguished from their normal litter mates by their smaller overall size, shorter hind limbs and shorter tail (Eicher *et al.*, 1976).

The cusp tips of mouse molars are not normally covered by enamel (Gaunt, 1956) so that any extension of a pulp horn through the full thickness of dentine should be visible externally. Furthermore, because lack of enamel promotes rapid initial attrition, a thin layer of dentine separating an elongated pulp horn from the mouth would soon be worn away, with consequent exposure of the pulp. Of 17 *Hyp*/*Y* dentitions examined grossly under a low-power binocular microscope, 10 (59 per cent) showed one or more pulp exposures in the lower molars, particularly the first molar, whereas, of 36 *Hyp*/+ dentitions from mice of comparable ages, only 9 (25 per cent) showed similar lesions. These exposures were not observed in the upper molars of either *Hyp*/*Y* or *Hyp*/+ animals, and are never found in the dentitions of normal mice. The difference in frequency of mice affected by pulp exposure between mutant males (*Hyp*/*Y*) and heterozygous females (*Hyp*/+) was significant ($\chi_1^2 = 4.37$, $p < 0.02$), which is consistent with incomplete dominance of the mutant gene for this character.

Demineralized mandibles (either right or left) from hypophosphataemic males and normal male litter mates were embedded in paraffin wax and serially sectioned in the sagittal plane (Table 1). Sections were cut at 5 μm and stained with haematoxylin and eosin, 0.1 per cent Azure A or by the method of van Gieson. Among the molars of the apparently unexposed mutant group, an additional example of exposure was discovered when the sections were examined.

As reported by Iorio *et al.* (1979b), the molars of *Hyp*/*Y* males tended to have rather larger pulp chambers and a wider predentine band than +/*Y*

Table 1. Numbers of mandibles sectioned from mice of different genotypes and different ages

Genotype	5 weeks	10 weeks	20 weeks	Total
Hyp/*Y* with externally visible exposures	3	5	2	10
Hyp/*Y* without externally visible exposures	3	3	3	9
+/*Y*	3	3	3	9

controls, with mutant dentine showing prominent interglobular areas of deficient mineralization typical of the human condition (Archard and Witkop, 1966; Tracy *et al.*, 1971) and not found in normal mice. There was no evidence of a progressive change in the consequence of pulp exposure with age, mice of all three age groups showing pulp necrosis and periapical involvement (Fig. 1A,B). Only one of the exposed pulp horns provided any evidence of attempted repair by dentine bridge formation (Fig. 1C). Whether or not this lack of repair can be attributed to abnormal odontoblast activity in these animals is a matter for speculation, but mineralized tissue, either as a bridge at the exposure site or elsewhere in the pulp chamber or root canal, has been observed regularly following experimental pulp exposure in the rat (Paterson, 1976).

REFERENCES

Archard H. O. and Witkop C. J. 1966. Hereditary hypophosphataemia (vitamin D resistant rickets) presenting primary dental manifestations. *Oral Surg.* **22**, 184–193.

Bixler D. 1976. Heritable disorders affecting dentin. In: *Oral Facial Genetics* (Edited by Stewart R. E. and Prescott G. H.). Mosby, St Louis.

Bulfield G. 1981. Inborn errors of metabolism in the mouse. In: *The Biology of the House Mouse* (Edited by Berry R. J.). *Symp. zool. Soc. Lond.* **47**, 643–665.

Eicher E. M., Southard J. L., Scriver C. R. and Glorieux F. H. 1976. Hypophosphatemia: mouse model for human familial hypophosphatemic (vitamin-D-resistant) rickets. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4667–4671.

Gaunt W. A. 1956. The development of enamel and dentine on the molars of the mouse, with an account of the enamel-free areas. *Acta anat.* **28**, 111–134.

Iorio R. J., Bell W. A., Meyer M. H. and Meyer R. A. 1979a. Radiographic evidence of craniofacial and dental abnormalities in the X-linked hypophosphatemic mouse. *Ann. Dent.* **38**, 31–37.

Iorio R. J., Bell W. A., Meyer M. H. and Meyer R. A. 1979b. Histologic evidence of calcification abnormalities in teeth and alveolar bone of mice with X-linked dominant hypophosphatemia (VDRR). *Ann. Dent.* **38**, 38–44.

Marks S. C., Lindahl R. J. and Bowden J. W. 1965. Dental and cephalometric findings in vitamin D resistant rickets. *J. Dent. Child.* **32**, 259–265.

Paterson R. C. 1976. Bacterial contamination and the exposed pulp. *Br. dent. J.* **140**, 231–236.

Tracy W. E., Steen J. C., Steiner J. E. and Buist N. R. 1971. Analysis of dentine pathogenesis in vitamin D resistant rickets. *Oral Surg.* **32**, 38–44.

Plate 1.

Fig. 1. (A) Sagittal section through lower first and second molars of a 5-week hypophosphataemic male mouse, showing a normal healthy pulp for the second molar but total necrosis of the first molar pulp associated with exposure. van Gieson. (B) Sagittal section through mesial root apex of the first molar shown in (A), showing dense inflammatory infiltration. van Gieson. (C) Sagittal section through cusp tip of a lower second molar from a 10-week hypophosphataemic male mouse, showing an attempt at dentine bridge formation. Azure A.

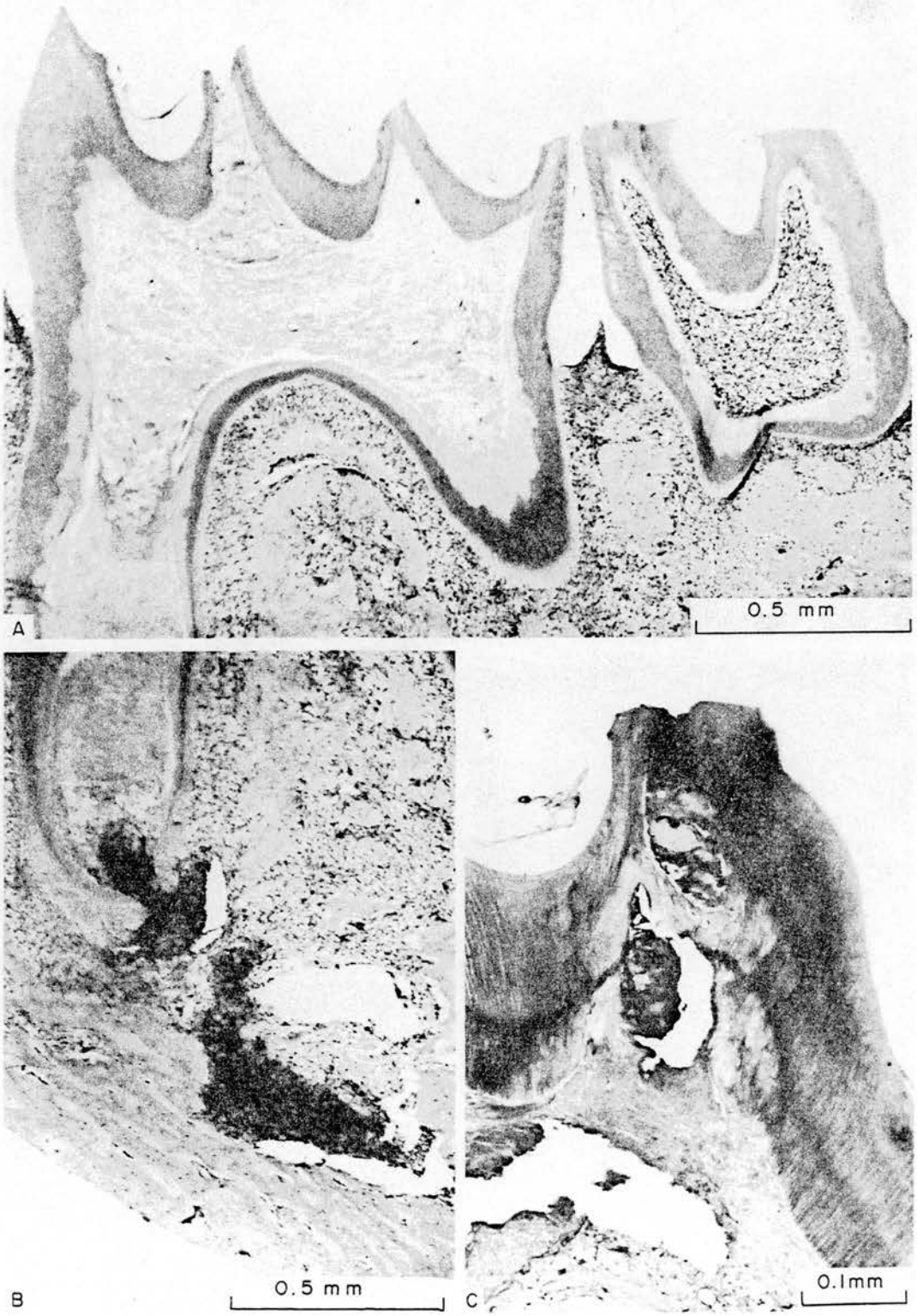


Plate 1.

EXPERIMENTAL ANALYSIS OF SINGLE GENE EFFECTS

One of the most prominent abnormalities shown by the mouse mutant 'downless', in addition to its effects on the dentition, is absence of hairs on the tail due to failure of hair follicle initiation. Normal embryonic tail epidermis recombined with either normal or mutant dermis produced hair follicles in culture whereas mutant epidermis with either normal or mutant dermis did not. The effect of 'downless' therefore appears to be restricted to the epidermal component of the system. Similar culture experiments using 'tabby' gave somewhat different results.

Reprinted from DEVELOPMENTAL BIOLOGY, Volume 34, No. 2, October 1973

DEVELOPMENTAL BIOLOGY **34**, 289–296 (1973)

Hair Follicle Initiation in Reciprocal Recombinations of *Downless* Homozygote and Heterozygote Mouse Tail Epidermis and Dermis

J. A. SOFAER

University of Cambridge, Department of Genetics, Milton Road, Cambridge, CB4 1XH, England

Accepted May 3, 1973

In the development of structures formed by the interaction of an epithelium and its underlying mesenchyme, the mesenchyme appears to be generally responsible for inducing the initiation of development. On the other hand, the epithelium must be competent to respond to the inductive stimulus if a structure is to be produced. One of the effects of the autosomal recessive mouse mutation *downless* is to suppress tail hair follicle initiation. Failure of initiation could therefore be due to failure in either the epidermal or the dermal component of the system, or both. Reciprocal recombinations between downless homozygote and heterozygote tail epidermis and dermis were made prior to the time when the first signs of follicle formation are visible in the tails of normal mice, and the recombined elements were allowed to continue growth and differentiation on the chick chorioallantoic membrane. The results suggest that the primary mutant effect is restricted to the epidermis. Explants composed of heterozygote epidermis with either heterozygote or homozygote dermis produced follicles, whereas explants composed of homozygote epidermis with either homozygote or heterozygote dermis did not.

INTRODUCTION

The semidominant X-linked gene tabby (*Ta*) in the mouse, and two recessive autosomal mimics of tabby, crinkled (*cr*) and downless (*dl*), are each associated with a mutant syndrome involving abnormalities of hair, teeth, and certain exocrine glands, all structures formed by the downgrowth of an epithelium into the underlying mesenchyme (Falconer *et al.*, 1951; Falconer, 1953; Grüneberg, 1965, 1966a,b, 1971; Sofaer, 1969a,b). Studies of a variety of similar structures in different species indicate that the mesenchyme is generally responsible for inducing the initiation of development and for the gross form of the structure that is produced (for example: Gomot, 1958; Dameron, 1961; Rawles, 1963; Kratochwil, 1969; Spooner and Wessells, 1970; Lawrence, 1971; Kollar, 1972), but that the epithelium may determine certain morphological or functional details (for example: Dhouailly, 1967; Sullivan, 1972; Lawson, 1972). The fundamental nature of mesenchymal induction is illustrated by the fact that it can operate across vertebrate classes. Mouse mesenchyme has induced feather initiation in chick corneal epithelium, an epithelium that normally produces no such structure (Coulombre and Coulombre, 1971).

However, the epithelium must have the capacity to respond to induction by the underlying mesenchyme if downgrowth is to occur. In the *scaleless* fowl, a mutant that has certain features in common with tabby, crinkled and downless mice (Abbott and Asmundson, 1957), the mutant effect has been shown by reciprocal recombination of normal and mutant epidermis and dermis to be intrinsic to the epidermis. Feathers and scales are formed by the interaction of normal epidermis with mutant dermis, but they do not develop from mutant epidermis in combination with normal dermis (Sengel and Abbott, 1963). The mutation therefore appears to affect the competence of the epidermis to react to a normal inductive stimulus. A further point of interest here is that, even though

the scaleless mutation primarily affects the epidermis, the dermis is not entirely normal, both in undissociated mutant skin (Goetinck and Sekellick, 1970) and in recombined skin composed of mutant epidermis and genetically normal dermis (Goetinck and Sekellick, 1972). This demonstrates that the interaction between epidermis and dermis is not in one direction only.

Studies on the coats of tabby and crinkled mice suggest that there is a timed gene effect expressed as suppression of new hair follicle formation between 12.5 and 17 days of gestation. The follicles that do form grow more slowly than normal (Falconer *et al.*, 1951), and there is a reduction in hair caliber and a lack of differentiation of the coat into hair types (Grüneberg, 1966b). It is during this period of suppression that hair follicles start to form in the tails of normal mice, the mesenchymal condensations that precede epithelial downgrowth appearing at about 16 days. The tails of the mutants are therefore generally bald. (Exceptions to this generalisation are provided by the alleles of tabby Ta^{J} and Ta^{c}, which produce abnormal hair sparsely distributed over the whole tail.) The original tabby allele (*Ta*), crinkled, and downless may each allow the presence of a few abnormal tail hairs, but on certain genetic backgrounds suppression of tail hair follicle initiation in mutant homozygotes and tabby hemizygotes appears to be complete. The tails of these mutants therefore provide both an opportunity to study the nature of dermal–epidermal interactions in the initiation of hair follicles, and a relatively simple system in which to investigate the ways in which the genes affect morphogenesis.

A comparison of hair follicle and hair development in culture between the body skin of normal and tabby mice has shown that tabby skin explanted from 13- and 14-day embryos retains its mutant characteristics (Hardy, 1969). Tabby skin at this stage of development therefore behaves autonomously when removed from the *in vivo* situation. However, on the question of whether the primary abnormalities produced by the mutant genes are restricted to either the epidermis or the dermis there has been no direct experimental evidence. Observations of *in vivo* development do perhaps tend to incriminate the epidermis, since the growth and differentiation of the epithelial component of the hair follicles, teeth and glands that do become initiated in the mutants are slower than normal (Falconer *et al.*, 1951; Sofaer, 1969a; Grüneberg, 1971). On the other hand, the interaction known to occur between epidermis and dermis leaves open the possibility that epidermal growth and differentiation are dependent on some critical factor or factors in the underlying dermis. It has in fact been suggested that tabby may act through cells of mesodermal origin, since the fine transverse banding pattern of the coat in tabby heterozygotes is reminiscent of the repetitive pattern of the somites in early development (Lyon, 1970). The present investigation is an attempt to determine experimentally whether the epidermis or the dermis is the primary site of activity of one of the mutant genes by making reciprocal recombinations between phenotypically normal and mutant tail epidermis and dermis and continuing growth in culture.

MATERIALS AND METHODS

Downless mice with a C3H/101 genetic background, obtained from the MRC Radiobiology Unit at Harwell, were the founder members of the mutant stock that was used. An autosomal gene was chosen in preference to tabby since two genotypes (*dl*+ and *dldl*) producing normal and mutant phenotypes could be obtained in the same litter. The tails of downless homozygotes of this stock are almost completely devoid of hair.

Downless heterozygote females were caged with downless homozygote males and were examined for vaginal plugs on the

following morning. The day on which a plug was found was regarded as day zero. On day 14 of gestation, 2 days before the first signs of follicle formation in normal tails, pregnant females were sacrificed by cervical dislocation and the embryonic litters were dissected out in Tyrode's solution containing 1000 units of penicillin and 25 mg of streptomycin per liter. Downless heterozygotes in these embryonic litters were distinguished from homozygotes by the presence of a postorbital tubercle, the first external sign of a developing postorbital vibrissa. Downless heterozygotes invariably possess the tubercle whereas homozygotes do not. In addition, a number of litters that were wild type for downless (and for tabby and crinkled) were obtained in a similar way.

The middle third of the tail of each embryo was removed in Tyrode's solution and the epidermal epithelium was cut longitudinally from one end of this segment to the other. Clean cutting of the epithelium was achieved by passing a tungsten needle longitudinally through the segment of tail, beneath the epithelium, and by cutting down to the needle with a scalpel from the outside. The majority of tail segments, each about 2 mm long and 1 mm in diameter, were then transferred to 2% trypsin (Difco 1:250) in Tyrode's solution and maintained at approximately 4°C for about 1.5 hr. After returning to Tyrode's solution the epidermis was peeled from the core of each tail segment, and reciprocal recombinations were made between littermates. From the litters that were segregating for downless all four possible genotype combinations of epidermis/dermis were made: *dl+/dl+*, *dldl/dldl*, *dl+/dldl*, and *dldl/dl+*.

Each recombination was made by placing a core of known genotype on a piece of black Millipore filter about 3 mm square, and by easing onto the core the epidermis removed from the tail of a littermate of known genotype. The pieces of filter facilitated manipulation of the cores and recombined segments, and provided a dark background against which the thin epidermal component could be seen clearly. The recombined tail segments were then carried on the pieces of filter to the chorioallantoic membrane of hens' eggs that had been incubated for 8 days. Once on the membrane the filter could be removed easily from beneath a recombined segment without disturbing the relationship between the core and its overlying epidermal epithelium. Some of the wild type tail segments were treated with trypsin, and explants were made by reciprocal recombination between littermates in the same way. A few wild type tail segments were not treated and were cultured undissociated, with the core tissue exposed by incision of the epidermal epithelium placed in contact with the chorioallantoic membrane. After 8 days of incubation on the chorioallantoic membrane the explants were removed, fixed in Bouin's fluid, serially sectioned at 10 μm and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Of 149 explants made, 106 were successfully recovered and sectioned. Sections were examined for the presence of early hair follicles, distinguished by the typical bell-shaped basal morphology of the epithelial downgrowth and by a basal concentration of melanin. Table 1 shows the total numbers of explants of each genotype combination examined and the numbers of explants in which follicles at this stage of development were observed. Also shown are the average numbers of follicles found in the positive explants; that is, those containing at least one early hair follicle. The results are consistent with the hypothesis of restriction of the primary mutant effect to the epidermis. Follicles were formed by heterozygous epidermis with either heterozygous or homozygous downless dermis, but never by homozygous downless epidermis.

The general relationship of the explanted epithelium to the tissues of the tail

TABLE 1

THE TOTAL NUMBERS OF EXPLANTS EXAMINED, THE NUMBERS CONTAINING EARLY HAIR FOLLICLES, AND THE AVERAGE NUMBERS OF FOLLICLES FOUND IN THE POSITIVE EXPLANTS FOLLOWING: (A) EXPLANTATION OF WHOLE MIDDLE THIRD TAIL SEGMENTS; AND (B) TRYPSIN DISSOCIATION AND RECIPROCAL RECOMBINATION OF EPIDERMIS AND DERMIS BETWEEN LITTERMATES

	(A) Undissociated	(B) Trypsin dissociation and reciprocal recombination				
Genotype of epidermis	++	++	*dl*+	*dldl*	*dl*+	*dldl*
Genotype of dermis	++	++	*dl*+	*dldl*	*dldl*	*dl*+
Total number of explants	7	14	20	21	24	20
Explants containing early follicles	7	6	8	0	6	0
Average follicle number in positive explants	23.0	2.0	4.5	—	2.7	—

core was variable. In some explants of all genotype combinations, the epithelium remained external to and in good contact with the core tissues, as the *in vivo* situation. In others, only of the dissociated group [Table 1 (B)], the explanted epithelium was only partly in contact with tail core tissue, the remainder spreading over or being incorporated alone in the neighboring chick chorioallantoic membrane. In the majority of explants of both the dissociated and undissociated groups at least some of the mouse epidermal epithelium had become included in the core tissues and had formed epithelial pearls or cysts of various sizes. Follicles were formed where the explanted epithelium came into contact with tail core tissue whether the epithelium remained external or not. Follicles were not observed in areas where the explanted epithelium appeared to be in contact only with tissues of the chick membrane. Nonspecific proliferative downgrowths of epidermal epithelium into the tissues of the tail core were a common finding.

Figure 1A shows the appearance of downless heterozygote tail skin at the time of explantation. Homozygous downless tail skin at this stage has a similar appearance. Figure 1B shows the separation between 14-day tail epidermis and dermis achieved by trypsin treatment. Figure 1C illustrates the state of tail hair follicle development 8 days later *in vivo* in a downless heterozygote. This can be compared with Fig. 1D, the appearance of tail skin at the same stage in a downless homozygote. Figure 1E, F, G, and H show examples of early follicles produced after 8 days on the chick chorioallantoic membrane by different explants of the *dl*+/*dldl* epidermis/dermis combination.

Among the explants made by dissociation and reciprocal recombination, the genotype combinations that produced follicles showed a low yield relative to the undissociated controls, both in terms of the proportion of explants containing follicles (about one-third overall), and in terms of the average number of follicles contained by the positive explants. The low follicle yield associated with downless heterozygote epidermis is not attributable to incomplete dominance of the wild type allele in culture, since the homozygous wild type recombinations showed a similarly low yield. Comparison between groups (A) and (B) in Table 1 indicates that the low follicle yield was due, at least very largely, to the dissociation and recombination procedure.

Despite the low follicle yield that occurred after dissociation and recombination, the overall pattern of the findings suggests that the downless mutation results in a failure of epithelial competence to react to a normal inductive stimulus. Evidence other than direct observation of the phenotype is thus provided for a similarity between the scaleless mutation in

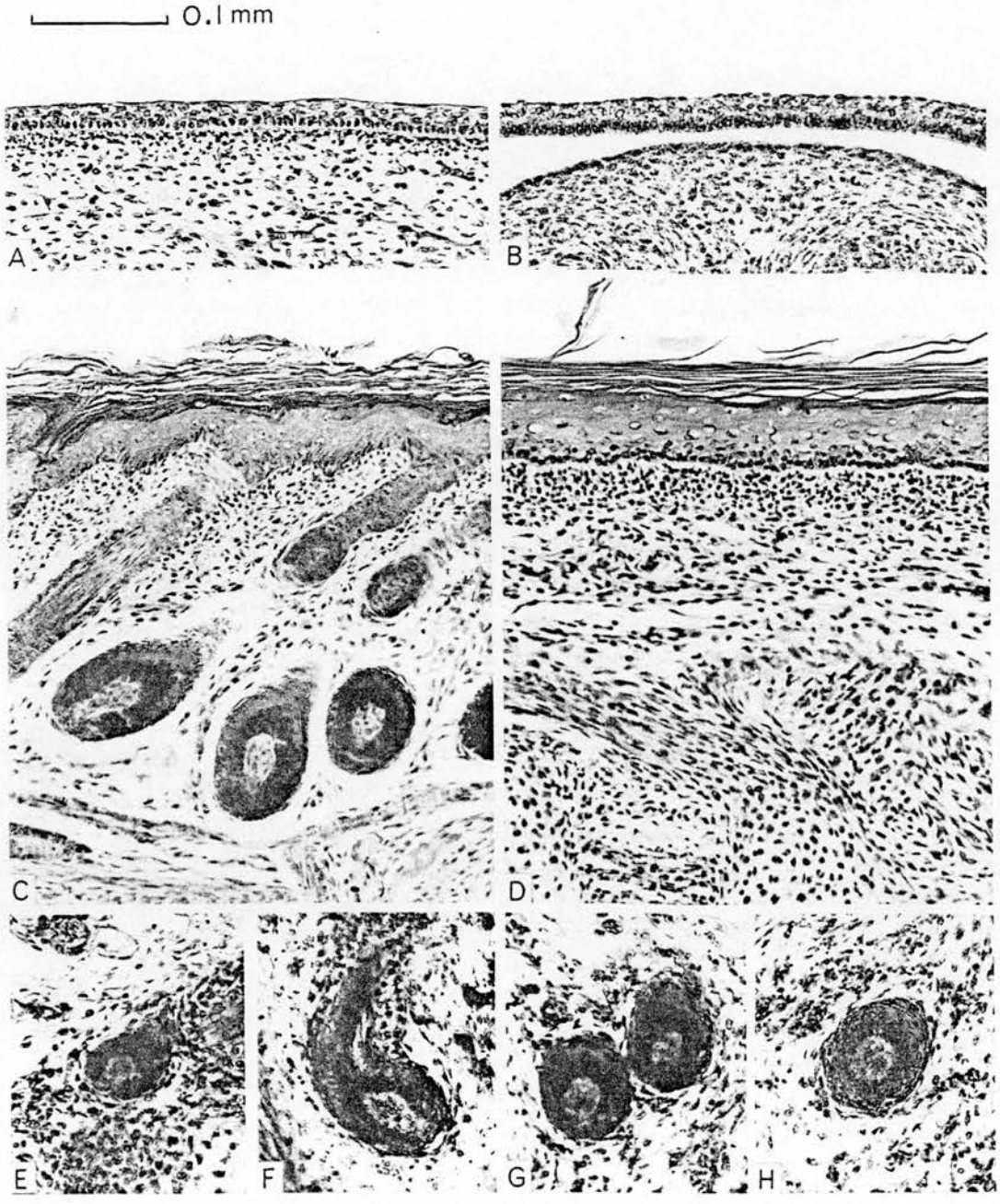


FIG. 1. (A) Undissociated 14-day embryonic downless heterozygote tail skin. Longitudinal section. (B) Separation between 14-day downless heterozygote tail epidermis and dermis achieved by trypsin treatment. Transverse section. (C) The state of tail hair follicle development *in vivo* 8 days later in a downless heterozygote. Longitudinal section. (D) Tail skin in a downless homozygote at the same stage as C. Longitudinal section. (E, F, G, H) Early hair follicles produced after 8 days on the chick chorioallantoic membrane by different explants of the *dl+/dldl* epidermis/dermis combination.

All tissue fixed in Bouin's fluid, sectioned at 10 μm and stained with hematoxylin and eosin.

the fowl and downless in the mouse. The epidermis of birds and mammals can therefore be affected by similar mutations leading to analogous phenotypic defects of

the cutaneous appendages. It is even possible that the wild type alleles of scaleless and downless (or tabby or crinkled) are evolutionarily homologous, and that more detailed comparison between the mutants could ultimately provide evidence for or against such a relationship.

In any event, observations on the interaction between nonmutant epidermis and dermis in the development of integumentary derivatives are very similar in birds and mammals. In both, the morphogenetic influence of the dermis is much greater than that of the epidermis, even though the final appendages are formed largely by epidermal cells. In birds, dermis from a prospectively featherless region of the skin is unable to take part in feather morphogenesis, whereas epidermis from such a region, when combined with appropriate dermis, can respond as well as epidermis from a prospectively feathered region. Furthermore, the specificity of scale as opposed to feather development is determined largely by the dermis, and even the fundamental pattern of feather morphology is dermally controlled (see review by Sengel, 1971).

Many interesting parallels have been demonstrated in mammals. Combinations of dermis from prospectively hairy regions with epidermis from the plantar surface of the embryonic foot have produced hairs, whereas combinations of epidermis from prospectively hairy regions with plantar surface dermis have produced only heavily keratinizing epithelium. Similarly, dermal papillae of early tooth germs are able to induce lip furrow epithelium, and even plantar surface epidermis, to form teeth; whereas the enamel organ, the early epidermal component of tooth germs, becomes a stratified keratinizing epithelium when confronted with plantar surface dermis, although it retains its ability to grow down into the dermis. In addition, reciprocal recombinations between epidermis and dermis of tooth-bearing and non-tooth-bearing regions of the mouse mandible have shown that the positions in which teeth develop within the jaw are controlled by the dermis; and reciprocal recombinations between dermal papillae and enamel organs of prospective incisor and molar teeth have shown that it is the dermal component also that dictates the shape of the tooth that is formed (see review by Kollar, 1972).

The similarities between the avian and mammalian systems are likely to be more than superficial, since cultured aggregates of young chick skin cells that would normally go on to produce feathers do not do so either in the presence of older prospectively feather-producing chick embryo skin cells (Garber and Moscona, 1967) or in the presence of prospectively hair producing mouse embryo skin cells of the comparably older developmental stage (Garber *et al.*, 1968). Also, as already mentioned, dermis from one class can cooperate with epidermis from the other to form chimeric structures (Coulombre and Coulombre, 1971). These observations suggest that there may be metabolic features that are common to the epidermis–dermis interaction systems of birds and mammals.

Even though the epidermis appears to play a secondary and rather passive role, the scaleless and downless mutations show that the ability of the epidermis to respond to dermal control can be independent of more fundamental epidermal functions. They also show that at least one component of the epidermal response is independent of the inductive capacity of the dermis. By contrast, in the Brahma breed of chick, where a normally scale-producing area develops feathers, the abnormality seems to arise through a modification of both the inductive capacity of the dermis and the response of the epidermis, although it has not been possible to show that the abnormal phenotype is due to allele substitution at a single locus (Goetinck, 1967, 1971). Further analysis of

single mutations, and of experimental interactions between tissues of different mutants, could possibly provide the basis for a more detailed understanding of the developmental processes that take place during the formation of integumentary derivatives.

The author is grateful to Dame Honor Fell for a gentle introduction to culture techniques, and to Dr. Bruce Cattanach for the gift of downless mice. Financial support was in the form of a Nuffield Foundation Dental Research Fellowship and a Research Project Grant from the Medical Research Council.

REFERENCES

ABBOTT, U. K., and ASMUNDSON, V. A. (1957). Scaleless, an inherited ectodermal defect in the domestic fowl. *J. Hered.* **48,** 63–70.

COULOMBRE, J. L., and COULOMBRE, A. J. (1971). Metaplastic induction of scales and feathers in the corneal anterior epithelium of the chick embryo. *Develop. Biol.* **25,** 464–478.

DAMERON, F. (1961). L'influence de divers mésenchymes sur la différentiation de l'épithélium pulmonaire de l'embryon de poulet en culture *in vitro*. *J. Embryol. Exp. Morphol.* **9,** 628–633.

DHOUAILLY, D. (1967). Analyse des facteurs de la différentiation spécifique de la plume néoptile chez le canard et le poulet. *J. Embryol. Exp. Morphol.* **18,** 389–400.

FALCONER, D. S. (1953). Total sex-linkage in the house mouse. *Z. Indukt. Abstamm. Vererbungsl.* **85,** 210–219.

FALCONER, D. S., FRASER, A. S., and KING, J. W. B. (1951). The genetics and development of 'crinkled' a new mutant in the house mouse. *J. Genet.* **50,** 324–344.

GARBER, B., and MOSCONA, A. A. (1967). Suppression of feather morphogenesis in co-aggregates of skin cells from embryos of different ages. *J. Exp. Zool.* **164,** 351–362.

GARBER, B., KOLLAR, E. J., and MOSCONA, A. A. (1968). Aggregation *in vivo* of dissociated cells. III. Effect of state of differentiation of cells on feather development in hybrid aggregates of embryonic mouse and chick skin cells. *J. Exp. Zool.* **168,** 455–472.

GOETINCK, P. F. (1967). Tissue interactions in the development of ptilopody and brachydactyly in the chick embryo. *J. Exp. Zool.* **165,** 293–300.

GOETINCK, P. F. (1971). Genetic tests on the association of brachydactyly and ptilopody in the fowl. *J. Hered.* **62,** 28–30.

GOETINCK, P. F., and SEKELLICK, M. J. (1970). Early morphogenetic events in normal and mutant skin development in the chick embryo and their relationship to alkaline phosphatase activity. *Develop. Biol.* **21,** 349–363.

GOETINCK, P. F., and SEKELLICK, M. J. (1972). Observations on collagen synthesis, lattice formation and morphology of scaleless and normal embryonic skin. *Develop. Biol.* **28,** 636–648.

GOMOT, L. (1958). Interaction ectoderme-mésoderme dans la formation des invaginations uropygiennes des oiseaux. *J. Embryol. Exp. Morphol.* **6,** 162–170.

GRÜNEBERG, H. (1965). Genes and genotypes affecting the teeth of the mouse. *J. Embryol. Exp. Morphol.* **14,** 137–159.

GRÜNEBERG, H. (1966a). The molars of the tabby mouse, and a test of the 'single-active X-chromosome' hypothesis. *J. Embryol. Exp. Morphol.* **15,** 223–244.

GRÜNEBERG, H. (1966b). More about the tabby mouse and about the Lyon hypothesis. *J. Embryol. Exp. Morphol.* **16,** 569–590.

GRÜNEBERG, H. (1971). The glandular aspects of the tabby syndrome in the mouse. *J. Embryol. Exp. Morphol.* **25,** 1–19.

HARDY, M. H. (1969). The differentiation of hair follicles and hairs in organ culture. *Advan. Biol. Skin* **9,** 35–60.

KOLLAR, E. J. (1972). The development of the integument: spatial temporal, and phylogenetic factors. *Amer. Zool.* **12,** 125–135.

KRATOCHWIL, K. (1969). Organ specificity in mesenchymal induction demonstrated in the embryonic development of the mammary gland of the mouse. *Develop. Biol.* **20,** 46–71.

LAWRENCE, I. E. (1971). Timed reciprocal dermal-epidermal interactions between comb, mid-dorsal, and tarsometatarsal skin components. *J. Exp. Zool.* **178,** 195–210.

LAWSON, K. A. (1972). The role of mesenchyme in the morphogenesis and functional differentiation of rat salivary epithelium. *J. Embryol. Exp. Morphol.* **27,** 497–513.

LYON, M. F. (1970). Genetic activity of sex chromosomes in somatic cells of mammals. *Phil. Trans. Roy. Soc. London B* **259,** 41–52.

RAWLES, M. E. (1963). Tissue interactions in scale and feather development as studied in dermal-epidermal recombinations. *J. Embryol. Exp. Morphol.* **11,** 765–789.

SENGEL, P. (1971). The organogenesis and arrangement of cutaneous appendages in birds. *Advan. Morphog.* **9,** 181–230.

SENGEL, P., and ABBOTT, U. K. (1963). *In vitro* studies with the scaleless mutant. Interactions during feather and scale differentiation. *J. Hered.* **54,** 254–262.

SOFAER, J. A. (1969a). Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *J. Embryol. Exp. Morphol.* **22,** 181–205.

SOFAER, J. A. (1969b). Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. *J. Embryol. Exp. Morphol.* **22,** 207–227.

SPOONER, B. S., and WESSELLS, N. K. (1970). Mammalian lung development: interactions in primordium formation and bronchial morphogenesis. *J. Exp. Zool.* **175,** 445–454.

SULLIVAN, J. A. (1972). Effect of epidermal rotation on orientation of scales in the chick. *Develop. Biol.* **28,** 176–182.

Genet. Res., Camb. (1974), **23**, *pp.* 219–225
Printed in Great Britain

Differences between *tabby* and *downless* mouse epidermis and dermis in culture

By J. A. SOFAER*

University of Cambridge, Department of Genetics, Milton Road, Cambridge CB4 1XH

(*Received* 21 *January* 1974)

SUMMARY

The semi-dominant X-linked gene *tabby* (*Ta*) in the mouse, and one of its recessive autosomal mimics, *downless* (*dl*) each produces a mutant syndrome that includes absence of hairs on the tail due to failure of tail hair follicle initiation. However, whereas *downless* tails failed to produce hair follicles in culture on the chick chorioallantoic membrane, which is in keeping with the adult phenotype of both *downless* and *tabby* mice, *tabby* tails produced follicles at about 40 % of the control level. Furthermore, in contrast to previous findings for *downless*, the culture of mixed genotype epidermis-dermis combinations provided no evidence of a primary epidermal effect in *tabby*.

1. INTRODUCTION

The semi-dominant X-linked gene *tabby* (*Ta*) in the mouse, and two recessive autosomal mimics of *tabby*, *crinkled* (*cr*) and *downless* (*dl*), are each associated with a mutant syndrome that is characterized by abnormalities of hair, teeth and certain exocrine glands, all structures formed by the downgrowth of an epithelium into the underlying mesenchyme (Falconer, Fraser & King, 1951; Falconer, 1953; Grüneberg, 1965, 1966*a*, *b*, 1971; Sofaer, 1969*a*, *b*). In mutant mice some of these structures fail to form altogether, and those that do form are usually reduced in size and have an abnormal morphology. The normal alleles of *tabby*, *crinkled* and *downless* therefore appear to be concerned with the interaction between epithelium and mesenchyme that takes place during the development of such structures.

One of the most prominent mutant abnormalities is the absence of hairs on the tail due to failure of tail hair follicle initiation. The degree of failure to form tail hairs is influenced by genetic background, and, at least for *tabby*, by the particular mutant allele that occupies the *tabby* locus; but for certain genotypes tail hair follicle initiation appears to be completely suppressed, or at least very nearly so.

Failure to initiate a downgrowth of epithelium that would go on to form a hair follicle could be due to an abnormality in either the epidermis, the epithelial component of the system, or the dermis, the mesenchymal component; or perhaps

* Present address: University of Edinburgh, The School of Dental Surgery, Edinburgh EH1 1JA.

both. It has been suggested that *tabby* may act through the dermis, since the fine transverse banding of the coat in *tabby* heterozygotes is reminiscent of the repetitive pattern of the somites in early development (Lyon, 1970; Mintz, 1971). However, previous work indicates that in *downless* mice the primary effect of the mutation on tail hair follicle initiation is restricted to the epidermis. Cultured combinations of $+/dl$ tail epidermis with dl/dl dermis produced follicles, whereas combinations of dl/dl epidermis and $+/dl$ dermis did not (Sofaer, 1973). The present paper describes a similar epidermis-dermis recombination experiment in which various combinations of Ta/Ta (or Ta), $+/dl$, and dl/dl tail epidermis and dermis were used. The experiment was undertaken to test independently the capacity of *tabby* epidermis and dermis to produce the mutant phenotype, and to compare the behaviour of *tabby* in culture with that of *downless*.

2. MATERIALS AND METHODS

The mutant stocks used were derived from *tabby* (original mutant allele *Ta*) and *downless* mice with a C3H/101 genetic background, obtained from the MRC Radiobiology Unit at Harwell. The degree of suppression of tail hair formation was determined for each stock by examining the tails of a sample of 50 adult mutant mice under a dissecting microscope for the presence of hairs in the distal, middle and proximal thirds of the tail. For comparison, the tails of 10 *downless* heterozygotes were also examined, and the mean number of hairs in the middle third of the tail was estimated. An estimate for each heterozygote was arrived at by counting the number of hairs per tail ring at the two extremes and at the centre of the middle third of the tail, and by taking the average of these three counts and multiplying by the number of middle third tail rings.

Timed matings were set up using Ta/Ta♀♀ with Ta♂♂, and $+/dl$ or dl/dl♀♀ with dl/dl♂♂. The day on which a vaginal plug was found was regarded as day zero. On day 14, two days before the first signs of hair follicle formation in normal tails, pregnant females were sacrificed and the embryonic litters were removed. Embryos from mixed litters of $+/dl$ and dl/dl individuals were classified on the basis of presence or absence of a postorbital tubercle, the first sign of a developing postorbital vibrissa. Heterozygotes invariably possess the tubercle whereas homozygotes do not .

The middle third of the tail of each 14-day embryo was dissected out in Tyrode's solution containing 1000 units of penicillin and 25 mg of streptomycin per litre, and the external epithelium was cut longitudinally from one end of this tail segment to the other. Segments to be used for epidermis-dermis recombination were then transferred to 2% trypsin (Difco 1:250) in Tyrode's solution and maintained at approximately 4° C for about $1\frac{1}{2}$ hours. After returning to Tyrode's solution the epidermal epithelium was peeled from the core of each tail segment, and recombinations were made between epithelium and tail cores of selected genotypes. The recombined tail segments were then carried to the chorioallantoic membrane of hen's eggs that had been incubated for

8 days. Undissociated control tail segments, not exposed to trypsin, were also explanted, with the core tissue exposed by incision of the epidermal epithelium placed in contact with the chorioallantoic membrane. Each egg received a single explant. After 8 days of incubation on the chorioallantoic membrane the explants were removed, fixed in Bouin's fluid, serially sectioned at 10 μm and stained with haematoxylin and eosin. The serial sections were then examined, and the number of early hair follicles contained in each explant was recorded.

A few additional details of the explantation technique are given in Sofaer (1973).

3. RESULTS

Table 1 shows the distribution of numbers of mutant tails of the two stocks according to the number of hairs in the distal, middle and proximal thirds of the tail. Suppression of hair formation was not complete for both *dl/dl* and *Ta/Ta* or *Ta* tails, and the incidence and position of hairs were very similar in the two mutants on this genetic background. When hairs did occur they tended to do so towards the distal rather than the proximal end of the tail.

Table 1. *The distribution of numbers of mutant tails according to the number of hairs in the distal, middle and proximal thirds of the tail*

Geno-type	Total tails	Position	Number of hairs					
			0	1–5	6–10	11–20	21–40	> 40
dl/dl	50	Distal $\frac{1}{3}$	37	8	3	0	2	0
		Middle $\frac{1}{3}$	44	5	0	1	0	0
		Proximal $\frac{1}{3}$	49	0	1	0	0	0
Ta/Ta or *Ta*	50	Distal $\frac{1}{3}$	36	11	2	1	0	0
		Middle $\frac{1}{3}$	46	4	0	0	0	0
		Proximal $\frac{1}{3}$	50	0	0	0	0	0

Table 2 compares $+dl$, *dl/dl*, and *Ta/Ta* or *Ta* adult tail phenotypes with the mean numbers of early hair follicles found per cultured undissociated embryonic tail segment. Also shown are adjusted means of hair and follicle number based on corresponding $+/dl$ values of 1. In both *dl/dl* and *Ta/Ta* or *Ta* adults the incidence of middle third tail hairs was very low compared with the $+/dl$ controls (around 1/10000 of the $+/dl$ level). However, whereas *dl/dl* explants produced no follicles at all, as was expected, *Ta/Ta* or *Ta* explants produced follicles at about 40% of the $+/dl$ control explant level. *Tabby* and *downless* therefore clearly responded in different ways to the culture system.

The results for trypsin dissociated and recombined explants of different genotype combinations are given in Table 3. Comparison with Table 2 shows that, in the change from culturing undissociated tail segments to trypsin dissociation and recombination prior to explantation, the proportion of follicle containing explants of $+/dl$ tail segments dropped from 100 % (19/19) to 40% (8/20), and that of *Ta/Ta* or *Ta* tail segments dropped from 82% (18/22) to 22% (5/23). The dis-

sociation and recombination procedure therefore affected both genotypes that produced follicles in this system in similar ways. Explants of *dl/dl* tail segments failed to produce follicles both when cultured undissociated (Table 2) and when cultured following trypsin dissociation and recombination (Table 3).

The data in Table 3 indicate that *downless* has its primary effect on the epidermis. All explants containing *dl/dl* epidermis failed to produce follicles (columns 3–5), whereas explants containing *dl/dl* dermis (except in combination with *dl/dl*

Table 2. *The mean number of hairs per middle third of adult tails, and the mean number of follicles found in cultured undissociated middle third explants*

	+/*dl*			*dl/dl*			*Ta/Ta* or *Ta*		
	N	*M*	*R*	*N*	*M*	*R*	*N*	*M*	*R*
Adult tails	10	2600 approx.	1	50	0·44	0·00017	50	0·14	0·00005
Explants	19	22·8	1	21	0	0	22	8·6	0·38

N is the number of tails or explants examined, *M* is the mean number of hairs or follicles, and *R* is the mean number of hairs or follicles relative to a *downless* heterozygote value of 1. All the +/*dl* explants, and 18 of the 22 *Ta/Ta* or *Ta* explants contained follicles. Means were based on the total number of tails or explants of each genotype examined.

Table 3. *The total numbers of explants of each epidermis/dermis genotype combination, the numbers containing hair follicles, and the average numbers of follicles found in the positive explants*

Column ...	1	2	3	4	5	6	7	8	9
Epidermis ...	+/*dl*	+/*dl*	*dl/dl*	*dl/dl*	*dl/dl*	*Ta*	*Ta*	*Ta*	+/*dl*
Dermis ...	+/*dl*	*dl/dl*	+/*dl*	*dl/dl*	*Ta*	+/*dl*	*dl/dl*	*Ta*	*Ta*
Total explants	20	24	20	21	25	23	24	23	21
Explants with follicles	8	6	0	0	0	11	9	5	4
Average follicles per positive explant	4·5	2·7	—	—	—	3·0	3·0	1·8	2·3
Positive:negative explants	14:30					20:27		9:35	

Ta refers to either *tabby* female homozygotes or *tabby* hemizygous males. The first four columns contain previous data (Sofaer, 1973).

epidermis – columns 2 and 7) showed some follicle yield. Explants containing *Ta/Ta* or *Ta* epidermis produced follicles (columns 6–8), and those containing *Ta/Ta* or *Ta* dermis (except in combination with *dl/dl* epidermis – columns 8 and 9) produced follicles also. This is consistent with the lack of complete mutant expression shown by cultures of undissociated *Ta/Ta* or *Ta* tails (Table 2).

Since undissociated *tabby* tail segments produced follicles at less than the control (+/*dl*) level it follows that some effect of *Ta* may be retained in the culture system and that it might be detectable in dissociated and recombined explants also. Thus, if *Ta* affects either the epidermis or the dermis, it may be possible to demonstrate a quantitative difference of follicle yield between explants containing

Ta epidermis as opposed to those containing *Ta* dermis. If *Ta* has its primary effect on the epidermis, as seems to be the case with *dl*, then explants containing *Ta* epidermis could show a lower follicle yield than those containing *Ta* dermis. Table 3 suggests that there is a tendency for the reverse to apply. There was a higher proportion of follicle containing explants among explants containing *tabby* epidermis (excluding those that also contained *tabby* dermis – columns 6 and 7) than among those containing *tabby* dermis (excluding those that also contained *dl*/*dl* or *Ta* epidermis – column 9); and the proportion for *tabby* epidermis (columns 6 and 7) was not significantly different from that for *downless* heterozygote control epidermis (columns 1 and 2). There is therefore some basis for combining columns 8 and 9 to give an overall indication of the effect of *tabby* dermis. The difference between the ratios of positive to negative explants for *tabby* epidermis (columns 6 and 7) as opposed to *tabby* dermis (columns 8 and 9) is of borderline significance. However, the difference between column 6 and column 9, and that between columns 8 and 9 as opposed to 1 and 2 are not significant. Nevertheless, while these results may be insufficient to implicate *tabby* dermis they do not provide any evidence of primary epidermal involvement in *tabby* mice.

4. DISCUSSION

The first difference disclosed between *tabby* and *downless* concerns their response to the culture system. Undissociated embryonic *downless* tail segments failed to produce hair follicles when cultured on the chick chorioallantoic membrane, whereas embryonic *tabby* tail segments produced follicles at about 40 % of the control level. There are two kinds of explanation for this. It may be that the *tabby* mutation results in a deficiency that is partly made good by the developing chick egg. The model proposed by Dun (1959) to explain the action of *tabby* is in fact based on a partial deficiency hypothesis. Alternatively, *tabby* may have its effect by inhibition of the normal developmental process. If this were the case, incomplete mutant expression could result from dilution of the inhibitory influence by the relatively massive volume of the chick egg.

Both the hypothesis of making good a deficiency and that of dilution of an inhibitor imply that a diffusible substance is involved in producing the *tabby* phenotype. Evidence for a diffusible substance comes from the observation that both 'normal' and '*tabby*' areas of the coat of *Ta* ↔ + chimaeras contain both normal and mutant hairs, only the proportion of the *tabby* effect differing in the two types of area (Cattanach, Wolfe & Lyon, 1972). Furthermore, a study of tail ring patterns in mice heterozygous or chimaeric for *tabby* has shown that only the chimaeras have phenotypically mosaic tails; the suggested interpretation being that patch size in heterozygote tails is much smaller than in chimaeras and falls within the diffusion range of a hypothetical gene product (McLaren, Gauld & Bowman, 1973).

The implications for *downless* are as follows. If *downless* results in a deficiency the chick egg must be unable to supply the explant with what is missing. On the

other hand, if *downless* acts by inhibition the inhibitor substance must be either poorly diffusible or produced in great excess.

The mode of action of the two genes might become clearer if undissociated mutant tail segments were cultured in a completely artificial system, free from the unknown factors associated with chorioallantoic grafting. It has already been reported that, in the plasma clot system, hairs developing in skin that has been taken from a prospectively hair producing area of *tabby* embryos show the characteristic mutant pattern of developmental timing and hair morphology (Hardy, 1969). The reason why the mutant characteristics of the cultured tissue are retained in this instance but not in the case of tail segments grafted onto the chorioallantoic membrane may be associated with the relative volumes of the two culture systems and the presence of a circulation in the chorioallantoic case. Both these factors would tend to allow more rapid exchange of a diffusible substance in the chorioallantoic situation.

The second difference between *tabby* and *downless* concerns the site of activity of each gene. There seems to be little doubt that the primary effect of *downless* is restricted to the epidermis. By contrast, comparison of the follicle yield between explants containing *Ta* epidermis and those containing *Ta* dermis does not provide an equally clear cut answer for *tabby*.

In all respects studied so far the phenotypes of *dl/dl* and *Ta/Ta* or *Ta* mice are qualitatively indistinguishable. Furthermore, the levels of mutant effect produced by the two genes appear to react in similar ways to the same changes of genetic background (Sofaer, 1969*b*). Nevertheless, the two genes have been shown here to respond differently to culture on the chick chorioallantoic membrane. It is possible that the difference of response may simply reflect a quantitative difference in the production of a single inhibitor. This would not necessarily be detectable under normal circumstances if there were sufficient inhibitor in *tabby* mice to cause maximum suppression of tail hair follicle initiation, but might be disclosed in culture because of the postulated dilution effect associated with chorioallantoic grafting. It is conceivable that such a difference could arise as a consequence of the difference between autosomal and X-linkage. Alternatively, since it appears possible that they may have their primary effect in different tissues, the genes may not be mimics because they produce closely related biochemical blocks, but rather because they result in abnormalities in separate but cooperating components of a single developmental system.

The author is grateful to Dr Bruce Cattanach for the gift of *tabby* and *downless* mice, and to Professors J. M. Thoday and H. Grüneberg for helpful criticism. Financial support was in the form of a Nuffield Foundation Dental Research Fellowship and a Research Project Grant from the Medical Research Council.

REFERENCES

CATTANACH, B. M., WOLFE, H. G. & LYON, M. F. (1972). A comparative study of the coats of chimaeric mice and those of heterozygotes for X-linked genes. *Genetical Research* **19**, 213–228.

DUN, R. B. (1959). The development and growth of vibrissae in the house mouse with particular reference to the time of action of the tabby (*Ta*) and ragged (*Ra*) genes. *Australian Journal of Biological Sciences* **12**, 312–330.

FALCONER, D. S. (1953). Total sex-linkage in the house mouse. *Zeitschrift für indukt. Abstammungs-und Vererbungslehre* **85**, 210–219.

FALCONER, D. S., FRASER, A. S. & KING, J. W. B. (1951). The genetics and development of 'crinkled' a new mutant in the house mouse. *Journal of Genetics* **50**, 324–344.

GRÜNEBERG, H. (1965). Genes and genotypes affecting the teeth of the mouse. *Journal of Embryology and Experimental Morphology* **14**, 137–159.

GRÜNEBERG, H. (1966*a*). The molars of the *tabby* mouse, and a test of the 'single-active X-chromosome' hypothesis. *Journal of Embryology and Experimental Morphology* **15**, 223–244.

GRÜNEBERG, H. (1966*b*). More about the *tabby* mouse and about the Lyon hypothesis. *Journal of Embryology and Experimental Morphology* **16**, 569–590.

GRÜNEBERG, H. (1971). The glandular aspects of the *tabby* syndrome in the mouse. *Journal of Embryology and Experimental Morphology* **25**, 1–19.

HARDY, M. H. (1969). The differentiation of hair follicles and hairs in organ culture. In: *Advances in Biology of Skin*. Vol. IX. *Hair Growth*, pp. 35–60.

LYON, M. F. (1970). Genetic activity of sex chromosomes in somatic cells of mammals. *Philosophical Transactions of the Royal Society of London, Series* B **259**, 41–52.

MCLAREN, A., GAULD, I. K. & BOWMAN, P. (1973). Comparison between mice chimaeric and heterozygous for the X-linked gene *tabby*. *Nature* **241**, 180–183.

MINTZ, B. (1971). Clonal basis of mammalian differentiation. In *Control Mechanisms of Growth and Differentiation. Symposium of the Society for Experimental Biology*, no. XXV, pp. 345–370.

SOFAER, J. A. (1969*a*). Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *Journal of Embryology and Experimental Morphology* **22**, 181–205.

SOFAER, J. A. (1969*b*). Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. *Journal of Embryology and Experimental Morphology* **22**, 207–227.

SOFAER, J. A. (1973). Hair follicle initiation in reciprocal recombinations of *downless* homozygote and heterozygote mouse tail epidermis and dermis. *Developmental Biology* **34**, 289–296.

MORE GENERAL OBSERVATIONS OF DENTAL DEVELOPMENT

In man, the upper lateral incisors are sometimes congenitally absent. When absence occurs on one side only, the central incisor adjacent to the missing tooth tends to be larger than the central on the other side. In the mouse, compensatory changes of growth rate occur between the developing lower first and second molars such that the rate of growth of the two germs taken together remains fairly constant. Both these findings provide further evidence for interaction between neighbouring tooth germs during development. Patients having cleft lip with or without a cleft of the palate exhibit abnormally high levels of tooth size asymmetry, not only in the vicinity of the malformation itself but throughout the dentition. This generalised instability of development may be to some extent under genetic control, as cases with positive family histories show some signs of greater asymmetry than those with negative family histories.

[Reprinted from HUMAN BIOLOGY, February, 1971, Vol. 43, No. 1.]

DEVELOPMENTAL INTERACTION, SIZE AND AGENESIS AMONG PERMANENT MAXILLARY INCISORS

BY J. A. SOFAER,* C. S. CHUNG,** J. D. NISWANDER * AND D. W. RUNCK †

THE development of the dentition is an apparently well integrated process during which growing structures are presumably influenced by their surroundings. Thus it is likely that the status of a developing tooth is not independent of that of its neighbours, and that variation in the size of one tooth germ may be reflected in others. Experimental evidence from the mouse suggests that under some circumstances there can be compensatory interaction between developing teeth. If in a given segment the teeth which develop early are large then those which develop late tend to be small or absent, and vice versa (Grüneberg, 1951; Grewal, 1962; Van Valen, 1962b; Sofaer, 1969). A simple way to test the hypothesis that such interaction occurs in man is to compare sides within individuals. Here heredity and the general environment are expected to influence both sides equally, so that any differences between sides can be assumed to be due to chance local environmental effects only.

Consider the simplest case that could be used for such a test; a morphological segment composed of just two teeth on each side. If as a result of chance local differences the earlier developing tooth is larger on the right than on the left, then, according to the hypothesis, the later developing tooth will tend to be larger on the left than on the right. It follows that the situation most likely to yield detectable asymmetry in one of the teeth of such a segment is unilateral extreme reduction or absence of the other. This situation sometimes occurs in the upper incisor region. Lateral incisors are not infrequently congenitally missing or small and peg shaped. Furthermore, the central incisors are easily measured so that variation in their size can be studied conveniently. The present paper is concerned with this variation in relation to different

* Human Genetics Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014.

** School of Public Health, University of Hawaii, Honolulu, Hawaii 96822.

† School of Dentistry, Department of Oral Surgery, University of Minnesota, Minneapolis, Minnesota 55455.

conditions of the lateral incisors, and with some thoughts on the developmental basis for peg and missing teeth.

METHODS

Data were obtained on a population study of dental characteristics in Hawaii. In this study over 17,000 high school students, ranging in age from 11 to 20 years, were examined. Details of the study design and examination procedure are given by Chung et al. (1970).

Missing teeth and morphological anomalies detected by visual examination were recorded. Measurements of the greatest mesio-distal diameter of the upper central incisors were obtained directly in the mouth using a Helios caliper and were recorded to the nearest $\frac{1}{10}$ mm. No radiographic confirmation of missing teeth was obtained, but it is felt that for the upper lateral incisor a reasonably accurate assessment of congenital absence can be obtained from visual inspection, palpation and history.

FINDINGS

Data appropriate for the analysis were available for 13,734 individuals. The distribution of cases, classified according to side and type of lateral incisor anomaly, and the means of left and right central incisor width for each class, are shown in Table 1. Table 2 lists the mean of

TABLE 1

The number of cases (N) and the mean mesiodistal diameters of left (L) and right (R) central incisors in units of 1/10 mm for nine classes of individuals according to whether the lateral incisors were normal, peg shaped, or missing

LEFT LATERAL	RIGHT LATERAL Normal	Peg	Missing
Normal	N = 13,298 L = 85.96 R = 85.92	N = 55 L = 84.11 R = 84.11	N = 36 L = 88.08 R = 89.65
Peg	N = 88 L = 83.47 R = 83.34	N = 109 L = 83.10 R = 83.23	N = 27 L = 83.37 R = 83.33
Missing	N = 41 L = 88.13 R = 86.99	N = 22 L = 85.67 R = 84.99	N = 48 L = 85.98 R = 86.56

TABLE 2

Mean and standard error of $(R + L)$ according to the condition of both lateral incisors. Mean $= \Sigma(R + L)/N$ where R and L are one individual's right and left central incisor measurements in units of 1/10 mm, and where N is the number of individuals in each category

Lateral Incisor Combination	N	Mean ± Standard Error
Both normal	13,298	171.88 ± 0.09
One peg and one normal	143	167.35 ± 0.91
Both peg	109	166.33 ± 1.13
One missing and one peg	49	168.47 ± 1.51
One missing and one normal	77	176.34 ± 1.38
Both missing	48	172.54 ± 1.76

TABLE 3

Means and variation of $(R - L)/(R + L)$. $M.S. = \Sigma((R - L)/(R + L))^2/2N$. N is the number of individuals in each category $(= d.f.)$, and R and L are one individual's right and left central incisor measurements in units of 1/10 *mm*

A. MISSING VERSUS PEG AND NORMAL LATERALS

Side of Missing Lateral	Mean ± Standard Error	d. f.	M. S.	F
Neither	$(-24.05 \pm 6.90) \times 10^{-5}$	13,560	6.46×10^{-5}	—
Left	$(-551.82 \pm 140.41) \times 10^{-5}$	63	12.42×10^{-5}	1.92 **
Right	$(450.25 \pm 141.14) \times 10^{-5}$	63	12.55×10^{-5}	1.94 **
Both	$(331.49 \pm 152.89) \times 10^{-5}$	48	11.22×10^{-5}	1.74 **

B. PEG VERSUS NORMAL LATERALS

Side of Peg Lateral	Mean ± Standard Error	d. f.	M. S.	F
Neither	$(-24.69 \pm 6.96) \times 10^{-5}$	13,298	6.445×10^{-5}	—
Left	$(-77.27 \pm 89.94) \times 10^{-5}$	88	7.118×10^{-5}	1.10 (N. S.)
Right	$(-3.09 \pm 108.39) \times 10^{-5}$	55	6.462×10^{-5}	1.00 (N. S.)
Both	$(63.23 \pm 87.59) \times 10^{-5}$	109	8.363×10^{-5}	1.30 *

* $P \leqq .05$.
** $P \leqq .01$.

In Table A the "Neither" category contains 10 more cases than does Table 1. This is because there are included 10 cases with unilaterally peg shaped laterals for which the side of the anomaly was not specified.

the sum of right and left central incisor diameters for different combinations of lateral incisor conditions on the two sides. It shows that in all three combinations containing a peg lateral the central incisors were smaller than normal, whereas a missing lateral on one side (with a normal lateral on the other) was associated with larger central incisors than normal. In cases of bilaterally missing laterals the centrals were slightly but not significantly larger than normal.

Table 3 shows the means and variation of $(R - L)/(R + L)$, where R and L are one individual's right and left central incisor measurements. This variable is an expression of asymmetry corrected for size of the central incisors. It is more meaningful in terms of development than $R - L$, the simple uncorrected difference between sides. Comparison of the mean square for each abnormal situation with the normal (the "Neither" category) by a variance ratio test shows that central incisors were significantly more asymmetrical when there was a missing lateral, either on one side or on both (Table 3A). Peg laterals, however, were only associated with an increase of asymmetry of borderline significance in bilateral cases (Table 3B).

Results of a stepwise multiple regression of missing laterals, and of peg laterals, on age, sex, $R + L$ and $R - L$ are presented in Table 4. Here $R - L$ is used simply to indicate which central was the larger of the two. It is not a measure of asymmetry. The condition of the lateral incisors was scored $+1$ if the abnormality being analyzed was on the right, -1 if it was on the left and 0 if it was bilateral or if both laterals were normal. This allowed the detection of a relationship between $R - L$ and the side of the lateral incisor anomaly after the effects of age, sex and size of the central incisors had been removed. Age, sex and $R + L$ were not significantly related to the side of either defect; there was no significant association between $R - L$ and peg lateral score; but there was a highly significant positive association between $R - L$ and missing lateral score. Since $R - L$ is positive when the right central is larger than the left, this significant positive regression coefficient indicates that there was a definite tendency for central incisors to be larger on the side where the lateral was missing than on the side where the lateral was present and either normal or peg shaped.

The salient features of the results are therefore as follows. Peg laterals were associated with small central incisors whereas a missing lateral on one side was associated with large central incisors; centrals were more asymmetrical than normal in the presence of missing laterals;

and in cases of unilaterally missing laterals the central on the missing side was larger than the central on the other side.

DISCUSSION

Consider first how the findings relate to the original hypothesis of compensatory interaction. As far as unilaterally missing teeth are concerned the results are certainly compatible with the interaction expectation. However, the possibility of a measurement bias being involved in the production of the directional difference between central incisors must not be overlooked. Such a bias could have been introduced simply by

TABLE 4

The relationship of age (years), sex (♀ = 0, ♂ = 1), R + L and R — L (in units of 1/10 mm) to the side of the lateral incisor anomaly (anomaly on the right = + 1, anomaly on the left = — 1, no anomaly or bilateral anomaly = 0)

A. MISSING VERSUS PEG AND NORMAL LATERALS

	Regression Coefficient	t
Age	—.00068	—1.22
Sex	—.00022	— .12
R + L	—.00006	— .67
R — L	.00179	4.44 ***

B. PEG VERSUS NORMAL LATERALS

	Regression Coefficient	t
Age	—.00065	—1.02
Sex	—.00079	— .39
R + L	.00014	1.46
R — L	.00006	.12

*** $P < 0.001$

the difference between the two sides in physical conditions for placing the calipers when a lateral was missing on one side and present on the other. Two features of the results suggest that a measurement bias was not entirely responsible. Firstly, central incisors were barely and not significantly larger than normal when laterals were missing on both sides, but were significantly larger than normal when a lateral was missing

on one side only and the other side was normal (Table 2). If absence of a lateral tended to cause an upward bias in measurement of the adjacent central the sum of right and left central incisor diameters in the bilaterally absent cases would be expected to be larger than in the unilateral cases. Secondly, bilateral absence would be expected to facilitate measurement of both centrals and therefore reduce any component of asymmetry due to errors of measurement. What was found was a significant increase of asymmetry in these cases compared with the normal (Table 3A), suggesting reduced developmental stability in this situation (Van Valen, 1962a; Bader, 1965). Thus it seems reasonable to assume that the results reflect real biological effects.

Consider now the nature of lateral incisor variability as a whole, and its relationship to the size of the central incisors. Peg and missing teeth clearly have some aetiological factor or factors in common as they appear together in individuals and in families more frequently than would be expected by chance (Montagu, 1940; Mandeville, 1949; Table 1). However, there is evidence that the two conditions are to some extent autonomous: the incidence of "degenerate" laterals and the incidence of missing laterals do not appear to vary together from one race to another (Montagu, 1940); in the present sample there was no laterality in the occurrence of missing teeth, whereas, for a peg lateral on one side associated with a normal lateral on the other, peg laterals were significantly more common on the left than on the right (Table 1, $P < .01$); and peg laterals were associated with smaller centrals than normal, whereas unilaterally missing laterals were associated with larger centrals than normal (Table 2).

It seems reasonable to postulate, both a priori and because of this partial autonomy of missing and peg laterals, that initiation and subsequent growth of a tooth germ depend to some degree on different factors. (Initiation in this context refers to the onset of rapid growth and morphodifferentiation of the previously dormant permanent tooth primordium.) Initiation, for example, clearly depends on an adequate primordium, but it is doubtful whether any excess over and above the minimal primordial requirement would produce a larger tooth. Some suggested basic relationships involved in normal tooth development, showing the partial independence of initiation and subsequent growth, are illustrated in Fig. 1. It is assumed here that once initiated a tooth germ will progress to form some sort of tooth.

In making an interpretation of the observations it is important to

keep in mind the normal sequence of development of the upper anterior teeth. It seems that both the central incisor and canine are initiated before the lateral incisor (Logan and Kronfeld, 1933). The lateral incisor therefore depends for its initiation on what remains of any necessary local requirements, and, once initiated, must compete with already established neighbours. Consequently it is easy to understand how the laterals could be the first teeth to manifest effects of restriction of any kind. Table 5 shows how this sequence of development, together with the relationships illustrated in Fig. 1, can result in the findings summarized in Table 2.

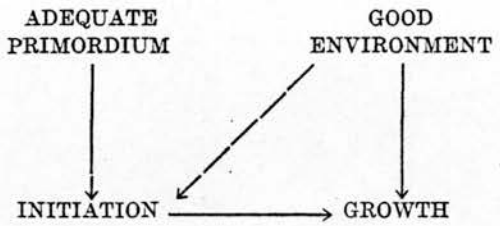


FIG. 1. SOME SUGGESTED BASIC RELATIONSHIPS INVOLVED IN NORMAL TOOTH DEVELOPMENT.

The relationship between Table 2 and Table 5 can be explained as follows. It is considered here that a peg lateral is always the product of a poor environment, whereas a normal lateral, not associated with a peg lateral on the opposite side, is the product of a good environment. Environment not only affects the laterals but the centrals also, so that a poor environment results in centrals that are smaller than normal. This accounts for the association between small centrals and peg shaped laterals. Cases of one missing and one normal lateral are each assumed to be due to an inadequate primordium in a good environment. Here, with the reduction in number of incisors from four to three, the centrals experience less competition for local requirements and consequently grow to a larger size than normal. When one lateral is missing and the other is peg shaped the environment is poor and possibly not sufficiently good to allow compensatory increase in size of the centrals. There is however some suggestion in Table 2 that the centrals in these cases were larger than in the bilaterally peg shaped cases. When only one lateral was missing and the other was normal the centrals were significantly larger than normal. When both laterals were missing the centrals were not significantly larger than normal. This is consistent with the assump-

tion that in all the unilateral cases the centrals developed in a good environment, as judged by the presence of one normal lateral, whereas in bilateral cases the missing teeth may be the product of either inadequate primordia or a poor environment, or both.

In connection with the adequacy of the local environment to allow initiation it is interesting to note that missing teeth have been associated with a generalized delay in dental development (Witkop, 1961; Garn, Lewis and Vicinus, 1963; Bailit and Sung, 1968). It may be that an undue delay between the initiation of the central and canine and the potential initiation of the lateral would, in the presence of a marginal environment, allow the established central and canine to become large enough effectively to suppress lateral initiation by overwhelming competition for restricted local requirements.

TABLE 5

The presumed origin of various conditions of the lateral incisors. A peg lateral always indicates a poor environment. + = adequate primordium or good environment. — = inadequate primordium or poor environment

Lateral Incisor Combination		Primordium	Environment
Both normal		+	+
One peg and one normal		+	—
Both peg		+	—
One missing and one peg		—	—
	or		
		+	—
One missing and one normal		—	+
Both missing		—	+
	or		
		+	—
	or		
		—	—

Consider now asymmetry of the central incisors. A convincing increase of asymmetry was only detected when lateral incisors were missing, not when there were peg laterals only. When a lateral was missing on one side the centrals were not only both larger than normal, but the central adjacent to the missing lateral was significantly larger than its counterpart on the other side. Thus both centrals appear to have reacted to the missing tooth by compensatory increase in size, the effect being

most marked in the central adjacent to the space. The increased asymmetry observed in cases of bilaterally missing teeth suggests that under normal circumstances environmental variation in the centrals may be buffered by interaction with the adjacent laterals. When there was a peg lateral on one side and a normal lateral on the other the inability to demonstrate asymmetry of the centrals can be attributed to all four teeth being present in a poor environment. Under such conditions severe competition presumably stifles the potential for compensatory increase in size of the central adjacent to the peg tooth. One final point that may be worth commenting on is the apparent tendency for the left central to be larger than the right when both laterals were normal, but for the right central to be larger than the left when both laterals were missing (Table 3). This presumably indicates some sort of developmental bias.

It can therefore be concluded that interaction does occur between developing human teeth, but that compensatory increase in size can occur only if requirements necessary for growth are not limited. It has also been suggested that, under certain conditions, large size of the central at a critical stage of development could be responsible for absence of the lateral. It is possible that the relationships that have been discussed are unique to the upper incisors. On the other hand, it may be that the variability observed at the distal ends of other morphological classes of teeth (Dahlberg, 1945) is due in part to interactions such as have been demonstrated here, the more distal later initiated germs tending to compensate for the combined deviation of their anterior neighbours from the norm.

SUMMARY AND ABSTRACT

The consequences of compensatory interaction between developing teeth of the same morphological class are most likely to be apparent when there is unilateral extreme reduction or absence of one of the members of this class. The expectation is then that the other teeth present on the affected side will be collectively larger than their counterparts on the normal side. This expectation can be tested conveniently in man in the upper incisor region. Lateral incisors are not infrequently congenitally missing or reduced, and the central incisors are easily measured. The results of such a test indicate that when a lateral was missing on one side the central adjacent to the missing tooth was larger than the central on the other side. Peg shaped laterals were associated with small central incisors and were regarded as being indicative of poor environmental conditions during development. These poor environmental conditions

were considered to be responsible for the inability to demonstrate a difference between centrals when there was a peg tooth on one side and the other side was normal, the potential for compensatory interaction being stifled by severe competition for restricted local requirements.

ACKNOWLEDGEMENT

The authors would like to thank Dr. P. L. Workman for helpful criticism.

LITERATURE CITED

BADER, R. S. 1965 Fluctuating asymmetry in the dentition of the house mouse. Growth, *29*: 291-300.

BAILIT, H. L. AND B. SUNG 1968 Maternal effects on the developing dentition. Arch. Oral Biol., *13*: 155-161.

CHUNG, C. S., D. W. RUNCK, J. D. NISWANDER, S. E. BILBEN AND M. C. W. KAU 1970 Genetic and Epidemiology Studies of Oral Characteristics in Hawaii School Children. I. Dental Caries, Periodontal Disease, Dental Eruption and Selected Dental Anomalies. (In Preparation).

DAHLBERG, A. A. 1945 The changing dentition of man. J. Am. Dent. Assoc., *32*: 676-690.

GARN, S. M., A. B. LEWIS AND J. H. VICINUS 1963 Third molar polymorphism and its significance to dental genetics. J. Dent. Res., *42*: 1344-1363.

GREWAL, M. S. 1962 The development of an inherited tooth defect in the mouse. J. Embryol. Exp. Morph., *10*: 202-211.

GRÜNEBERG, H. 1951 The genetics of a tooth defect in the mouse. Proc. Roy. Soc., B., *138*: 437-451.

LOGAN, W. H. G. AND R. KRONFELD 1933 Development of the human jaws and surrounding structures from birth to the age of fifteen years. J. Am. Dent. Assoc., *30*: 379-427.

MANDEVILLE, L. C. 1949 Congenital absence of permanent maxillary lateral incisors: a preliminary investigation. Ann. Eugen., *15*: 1-10.

MONTAGU, M. F. A. 1940 The significance of the variability of the upper lateral incisor teeth in man. Human Biol., *12*: 323-358.

SOFAER, J. A. 1969 Aspects of tabby-crinkled-downless syndrome. I. The development of tabby teeth. J. Embryol. Exp. Morph., *22*: 181-205.

VAN VALEN, L. 1962a A study of fluctuating asymmetry. Evolution, *16*: 125-142.

——— 1962b Growth fields in the dentition of Peromyscus. Evolution, *16*: 272-278.

WITKOP, C. J. 1961 Studies of intrinsic disease in isolates with observations on penetrance and expressivity of certain anatomical traits. *In* Congenital Anomalies of the Face and Associated Structures. Ed. by S. PRUZANSKY, Charles C. Thomas, Springfield, Ill.

Archs oral Biol. Vol. 22, pp. 71 to 72. Pergamon Press 1977. Printed in Great Britain.

COORDINATED GROWTH OF SUCCESSIVELY INITIATED TOOTH GERMS IN THE MOUSE

J. A. SOFAER
University of Edinburgh, School of Dental Surgery, Edinburgh EH1 1JA, Scotland

Summary—Dentitions are formed by the successive initiation of individual tooth germs, but there are periods of overlap, each new germ being initiated before the previous germ has reached its full size. It might be expected that dental growth as a whole proceeds at a particularly high rate during these overlap periods. However, measurement of mouse lower first and second molars at daily intervals from 14 to 23 days after fertilization showed that there were compensatory changes in growth rate of the two germs during their overlap period, and that the rate of growth of the two teeth taken together remained fairly constant.

During the development of a dentition, teeth take up an increasing proportion of the space provided by the growing jaw. It is therefore likely that factors in the local tissue environment of the jaw that are necessary for the growth of tooth germs are more fully used up as development progresses, and that any observable effect of restriction of these factors will be most pronounced in the last tooth to develop. Put more generally, variation in the local tissue environment is more likely to affect later developing teeth than their earlier developing neighbours. This is in keeping with the well-known greater variability of the later developing teeth within each morphological class (incisors, premolars and molars), the greater asymmetry and lower heritability of these later developing teeth (Sofaer, Bailit and MacLean, 1971; Alvesalo and Tigerstedt, 1974), and, at least in man, probably also the greater evolutionary reduction of the later developing teeth compared with their earlier developing neighbours (Sofaer, 1973).

Dentitions are formed by the successive initiation of individual tooth germs, but there are always periods of overlap, each new tooth being initiated before the previous tooth germ has reached its full size. It might therefore be expected that, superimposed on the progressive local environmental restriction that occurs with dental development, these periods of overlap may result in phases of particular sensitivity to the limitation of factors necessary for growth. The reason for this is that the rate of growth of the two teeth combined might be greater during a period of overlap than the growth rate of either the earlier developing tooth germ alone just before, or the later developing tooth germ alone just after, the period of overlap.

To investigate the nature of such a period of overlap, developing lower first and second molars were measured in routinely prepared 10μm sagittal sections of embryos and newborn mice from the inbred strain CBA/Cam. Material was fixed in Bouin's fluid at daily intervals from 14 to 23 days after fertilization (as determined by the presence of a vaginal plug), and the left sides only of a total of 10 mice (taken from at least three litters with different parents) were measured for each of these 10 developmental stages. The maximum anteroposterior diameter of each tooth germ, or at earlier stages each tooth primordium, and the mid-sagittal anteroposterior diameter of the head (snout to back of head, parallel to occlusal plane), were taken from images produced by a projection microscope at a standard magnification.

Table 1 lists the means and standard errors of these diameters. Over the 10 stages investigated, there was a more or less regular increase in size of the two teeth and of the head, except from 21 to 22 days. During this particular interval, there was no size increase in the first molar and the head, and a smaller increase in the second molar than in the immediately previous and subsequent day intervals. This interruption of growth occurred just after birth, and can therefore reasonably be attributed to the physiological adjustments necessary during the neonatal period.

Figure 1 shows mean tooth diameters (first molar, second molar, and the sum of the two) expressed as percentages of mean head diameter at the different developmental stages. From 14 to 16 days after fertilization, first and second molars grew, relative to head diameter, at about the same rate. From 16 to 18 days, the first molar rate increased and this was mirrored by a decrease in the second molar rate. This decrease was so marked that at 18 days the second molar was smaller, relative to head diameter, than during the previous two days. From 18 days, the first molar rate started to decrease slightly, and after 20 days was levelling off. This change was again mirrored by the second molar, where size increased at an accelerating rate between 18–23 days. The changes of growth rate in one tooth germ were so well balanced by those in the other that the rate of increase of the combined anteroposterior diameters of the two teeth, relative to head diameter, was almost perfectly constant over the period investigated. There was therefore no evidence for a critical overlap period when dental growth as a whole was proceeding at a particularly high rate.

One of the most interesting features of the results is the complementary change in growth rate of the first and second molar germs at 16 days. This was

Table 1. Means and standard errors of the maximum anteroposterior diameters of lower first and second molars and of the head between 14–23 days after fertilization. The left sides only of a total of ten mice were measured for each developmental stage

	Diameters in millimeters					
	First molar		Second molar		Head	
Day	Mean	s.e.	Mean	s.e.	Mean	s.e.
14	0.215	0.013	0.105	0.005	4.88	0.07
15	0.288	0.015	0.165	0.009	5.64	0.07
16	0.362	0.014	0.242	0.012	6.56	0.11
17	0.494	0.019	0.280	0.006	7.57	0.15
18	0.651	0.012	0.289	0.005	8.34	0.09
19	0.782	0.026	0.348	0.008	9.12	0.13
20	0.947	0.026	0.396	0.011	9.60	0.09
21	1.097	0.021	0.482	0.010	10.74	0.20
22	1.094	0.021	0.533	0.016	10.52	0.24
23	1.191	0.010	0.686	0.022	11.26	0.13

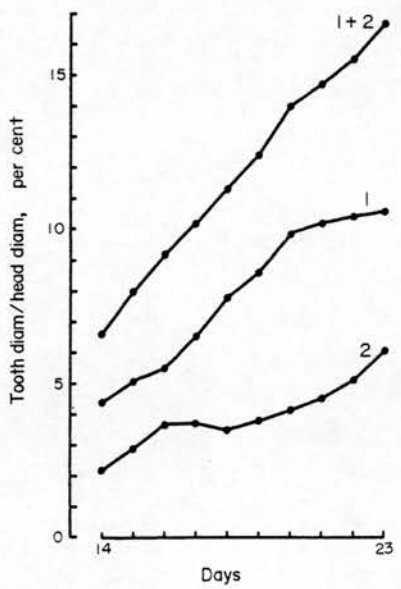


Fig. 1. Mean anteroposterior tooth diameters expressed as percentages of mean anteroposterior head diameter from 14 to 23 days after fertilization. 1 = first molar, 2 = second molar, 1 + 2 = sum of first and second molars.

not reported in a previous study, which, although carried out in great detail, recorded second molar growth from only 17 days (Gaunt, 1963). The present study shows that from 14 to 16 days first and second molar germs increased in size at approximately the same rate, suggesting similar levels of cellular activity in the two primordia at the early stages of development. This is in keeping with the postulated origin of all three molars of one quadrant in the mouse from a single cell mass (Lumsden and Osborn, 1976). During these early stages, the first and second molar primordia were closely associated with each other, with no clear evidence of separate origins for the two teeth. Nevertheless, despite this early close association and similar rate of growth, only the growth rate of the first molar increased at 16 days. The opposite effect in the second molar suggests some kind of competitive interaction between the two teeth. Growth rate in the second molar did not start increasing again until two days later, and the size of the second molar germ, relative to head diameter, had not recovered its 16-day level until three days later. This is consistent with the period of between two and three days that separates equivalent stages of histodifferentiation in the two teeth.

It therefore seems likely that lower first and second molars in the mouse develop from cells that, initially, show the same level of activity; but that, for some as yet unknown reason, a phase of rapid growth starts in the first molar germ, postponing a similar growth spurt in the second molar for between two and three days through the suppressive effect of competitive interaction. It is possible that the smaller size and simpler morphology of the second molar are simply consequences of this two-to-three day delay, cellular activity in the second molar germ being modified in some way by the period of suppression. Over the period investigated, interaction between the developing germs apparently results in a fairly constant rate of growth for the two teeth taken together, relative to head diameter, with no critical overlap period when their combined growth proceeds at a particularly high rate.

REFERENCES

Alvesalo L. and Tigerstedt P. M. A. 1974. Heritabilities of human tooth dimensions. *Hereditas* **77**, 311–318.

Gaunt W. A. 1963. An analysis of the growth of the cheek teeth of the mouse during ontogeny. *Acta anat.* **54**, 220–259.

Lumsden A. and Osborn J. W. 1977. Development of the mouse dentition in culture. British Division, Internat. Ass. for Dent. Res., Abstract. *J. dent. Res.* (in press).

Sofaer J. A. 1973. A model relating developmental interaction and differential evolutionary reduction of tooth size. *Evolution* **27**, 427–434.

Sofaer J. A., Bailit H. L. and MacLean C. J. 1971. A developmental basis for differential tooth reduction during hominid evolution. *Evolution* **25**, 509–517.

Archs oral Biol. Vol. 24, pp. 141 to 146
Pergamon Press Ltd 1979. Printed in Great Britain

HUMAN TOOTH-SIZE ASYMMETRY IN CLEFT LIP WITH OR WITHOUT CLEFT PALATE

J. A. SOFAER

Department of Oral Medicine and Oral Pathology, University of Edinburgh, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR, and Department of Human Genetics, Western General Hospital, Edinburgh EH4 2HU, Scotland

Summary—Failure of corresponding teeth on the right and left sides to form as exact mirror images of each other is an expression of imprecise developmental control. Levels of tooth-size asymmetry can therefore be used to quantify developmental instability in different regions of the dentition. Mesiodistal and buccolingual diameters of deciduous and permanent teeth were measured to the nearest 0.1 mm on serial plaster models of 77 patients with cleft lip with or without a cleft of the palate, and 63 control non-cleft orthodontic patients. In the cleft group as a whole, there were abnormally high levels of tooth-size asymmetry but, although most marked in the upper lateral incisor region, these were neither restricted to the vicinity of the cleft nor to the upper jaw. Thus, in addition to major local disturbances related to the malformation itself, it appears that tooth-size asymmetry results from a generally high level of developmental instability throughout cleft lip/palate dentitions. This generalized developmental instability may be to some extent under genetic control, as cases with positive family histories showed some signs of greater asymmetry than those with negative family histories.

INTRODUCTION

In clinic populations of patients having cleft lip with or without a cleft of the palate [CL(P)], less than 10 per cent suffer from syndromes of major malformations, whereas in more than 90 per cent the cleft occurs alone unaccompanied by any obvious associated abnormality. The majority of cases that occur as part of syndromes are caused by single mutant genes or chromosomal abnormalities. However, nearly all those where CL(P) occurs alone have a multifactorial aetiology; that is, several factors, some genetic and some environmental, contribute to the development of the malformation. In the multifactorial situation, the degree of genetic determination varies from one family to another, the evidence for this being the wide range in number of affected relatives found among families of cleft individuals (Fraser, 1970; Ross and Johnston, 1972; Gorlin, Pindborg and Cohen, 1976).

For practical purposes, it is useful to divide multifactorial cases into two classes: those with no affected relatives, the negative family history or sporadic class; those with at least one affected relative, the positive family history class. In both, genetic factors may contribute towards producing the malformation but, in the sporadic situation, the genetic influence is likely to be minimal, whereas, in the familial situation, the genetic component is on average relatively large.

Adams and Niswander (1967) suggested that the genetic component in multifactorial instances does not act positively to produce the malformation, but rather reduces the resistance of the developing individual to adverse environmental influences. They used the term buffering to convey the capacity of the individual to regulate its development, in keeping with Waddington (1957) and analogous to the more familiar usage of the term in discussions of physiological homeostatic mechanisms. Adams and Niswander considered the level of buffering to be an attribute of the developing individual as a whole, and therefore that reduced buffering should result in a generalized instability of development. They consequently suggested that a cleft only occurs in conjunction with reduced buffering because development in the future upper lateral incisor region is particularly sensitive to environmental effects. If this is so, those who have inherited a low level of buffering require only minor environmental insults to produce the malformation, whereas in those with near normal buffering, a cleft is only produced by a severe adverse environmental influence.

One of the best general indicators of developmental instability is the asymmetry of bilaterally paired structures. It is assumed that the same genetic and general environmental factors control development on the two sides of the body, so that the extent to which the sides differ is a measure of the lack of precision of this developmental control. Therefore, as buffering is reduced, asymmetry increases. In studies of sporadic and familial CL(P) without associated malformations (Adams and Niswander, 1967; Woolf and Gianas, 1976, 1977), it has been shown that abnormally high levels of asymmetry occur in structures remote from the cleft, both in familial cases and their non-cleft relatives. However, no abnormality of asymmetry has been demonstrated in sporadic cases or their relatives. The variables used to express asymmetry were the buccolingual diameter of the lower first molar and three dermatoglyphic characteristics: the *atd* angle, finger-ridge count and fingerprint pattern. The genes common to familial CL(P) cases and their non-cleft relatives therefore appear to increase asymmetry in parts of the body remote from the cleft, indicating an unusually high inherited level of developmental

Table 1. The numbers of individuals investigated

Controls	CL(P) FH − ve	FH + ve	FH unknown	Total CL(P)
63	39	14	24	77

instability in these individuals. These observations therefore support the hypothesis that the genetic component in the multifactorial situation does not act directly to produce the malformation itself, but indirectly, through a reduction in buffering.

There are thus two groups of individuals suffering from the same malformation. The familial group shows signs of an inherited generalized instability of development: it is therefore likely that in this group abnormally high levels of asymmetry occur throughout the dentition, in addition to more severe local disturbances related to the cleft itself. However, in the sporadic group, without generalized instability, the abnormal levels of asymmetry are likely to be due only to local effects and therefore restricted to the region around the cleft.

The aim of my study was to assess the effects of local and general influences on tooth-size asymmetry in CL(P) dentitions and, more specifically, to compare the observed patterns of asymmetry with those expected on the basis of the reduced buffering hypothesis.

MATERIALS AND METHOD

All CL(P) cases seen at Edinburgh Dental Hospital's Orthodontic Department in recent years, for whom both serial plaster models and general hospital records (Royal Hospital for Sick Children, Edinburgh) were available, were included. The general hospital records were studied to ascertain details of the family histories and the cases were divided into: those with a positive family history (FH + ve), having at least one affected blood relative; those with a negative family history (FH − ve), having no affected blood relative recorded; those for whom the information given in the hospital records was considered insufficient to classify either one way or the other (FH unknown). Plaster models from a randomly-selected group of non-cleft orthodontic patients served as controls. Table 1 shows the numbers of individuals studied.

Mesiodistal and buccolingual diameters were measured to the nearest 0.1 mm using dial calipers for all deciduous and permanent teeth present, except those for which caries, restorations or incomplete eruption would have rendered measurement inaccurate. Mesiodistal and buccolingual axes were determined with reference to the anatomy of each tooth, making them independent of tooth alignment, and the measurements taken were the maximum supragingival crown diameters along these axes. The presence of caries, restorations and incomplete eruption, as well as the quality of the cast, were taken into account when selecting the best cast for measurement in instances where a tooth appeared on more than one cast of the same subject. In three CL(P) cases, there was an additional tooth in the upper lateral incisor region on one side, one in the deciduous and two in the permanent dentition. For these subjects, the tooth judged to be the least normal in appearance was not included in the analysis.

The measure of asymmetry used, V, was the variance of the difference between sides adjusted for tooth size. That is, the variance of $(R - L)/(R + L)$, where R and L are corresponding measurements on the right and left sides. For each diameter and tooth, asymmetry was expressed as either the ratio of the total cleft-group asymmetry–variance to the corresponding control variance, or the ratio of the familial cleft-group asymmetry–variance to the corresponding variance for the sporadic group. Variance ratios were tested for significant difference from unity, using the standard F test. The numbers of right–left tooth pair measurements available for the calculation of the asymmetry variances, many of them considerably lower than the numbers of individuals in the different groups, are shown in Table 2.

EXPECTATIONS AND RESULTS

Figure 1 shows two possible patterns of the asymmetry ratio in the dentition as a whole. As the vari-

Table 2. The numbers of right–left tooth pairs available for measurement in all cleft cases (CL(P)), familial cleft cases (FH + ve), sporadic cleft cases (FH − ve) and controls

		Tooth pair A	B	C	D	E	1	2	3	4	5	6	7
Upper	CL(P)	40	21	53	46	47	48	5	26	34	29	55	23
	Control	13	12	13	11	13	56	52	50	36	51	51	37
Lower	CL(P)	36	43	59	47	46	61	60	44	38	22	55	20
	Control	11	12	13	12	9	55	56	52	41	47	50	40
Upper	FH + ve	8	3	11	10	9	9	0	2	5	4	8	4
	FH − ve	23	14	27	24	25	23	4	13	14	11	27	10
Lower	FH + ve	10	11	13	9	9	10	10	7	7	3	10	0
	FH − ve	20	24	30	25	24	30	29	20	18	12	28	10

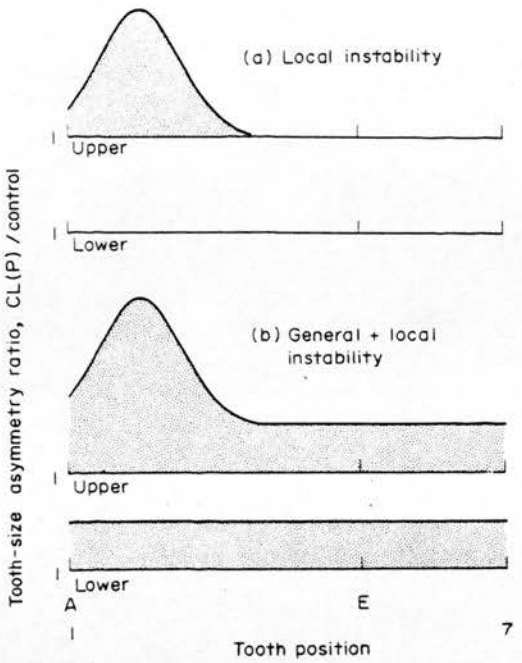


Fig. 1. Two possible patterns of the asymmetry ratio in the dentition as a whole.

able plotted is a ratio, the value 1 indicates that the cleft group and control group asymmetry variances are the same. Figure 1a shows the pattern that might be expected if tooth-size asymmetry in CL(P) were entirely the result of local disturbances related to the malformation itself. There is a peak of the asymmetry ratio in the upper lateral incisor region, with asymmetry in the more distal teeth of the upper jaw, and in all teeth of the lower jaw, at normal control levels. Figure 1b shows what might be expected if a generalized reduction of buffering capacity were associated with CL(P). There is a moderate increase in asymmetry throughout the dentition, with a superimposed peak in the upper lateral incisor region.

Figures 2 and 3 show the actual patterns of results in the deciduous and permanent dentitions for all CL(P) cases, irrespective of family history, compared with controls. Asymmetry here is expressed as the log of the asymmetry ratio. Zero therefore indicates equality of cleft and control asymmetry, positive values indicate that cleft asymmetry was greater than control asymmetry, and negative values that cleft asymmetry was less than control asymmetry. The log transformation was used simply to give equal weight on the histograms to situations where the untransformed asymmetry ratio was greater or less than unity.

Figure 2 shows the results for deciduous teeth. In the upper jaw, CL(P) cases were clearly more asymmetrical than controls in the incisor region, with some suggestion of increased asymmetry more distally. Only one measurement showed less asymmetry in the cleft group than in controls, and this difference was not significant. In the lower jaw, there was also only one measurement that showed less asymmetry in the cleft group and, of the rest that showed greater asymmetry in the cleft group, three were significantly more asymmetrical than the controls.

In the permanent dentition (Fig. 3), the results were similar but much more definite. In the upper jaw, every measurement showed greater asymmetry in the cleft group, and for only one did the difference fail to reach significance. The ratio of cleft to control values was clearly greatest at and towards the lateral incisor region. In the lower jaw, 12 of the 14 measurements showed greater asymmetry in the cleft group and, for six, the differences were significant.

Figure 4 shows possible patterns of the asymmetry ratio in the upper jaw only, in keeping with the hypothesis that a generalized reduction of buffering occurs in familial but not sporadic cases. Figure 4a shows the pattern when asymmetry is due only to local disturbances caused by the cleft itself. This is the expectation for the ratio of FH − ve to control asymmetry. Figure 4b shows the pattern when there is a generalized reduction of buffering, with superimposed local effects due to the malformation itself. This is the expectation for the ratio of FH + ve to control asymmetry. Figure 4c shows the pattern for the ratio of FH + ve to FH − ve asymmetry, based on the familial and sporadic expectations shown in Figs 4a and 4b. In Fig. 4c, the ratio is close to unity in the incisor region, but consistently higher more distally. A consistent difference between familial and sporadic

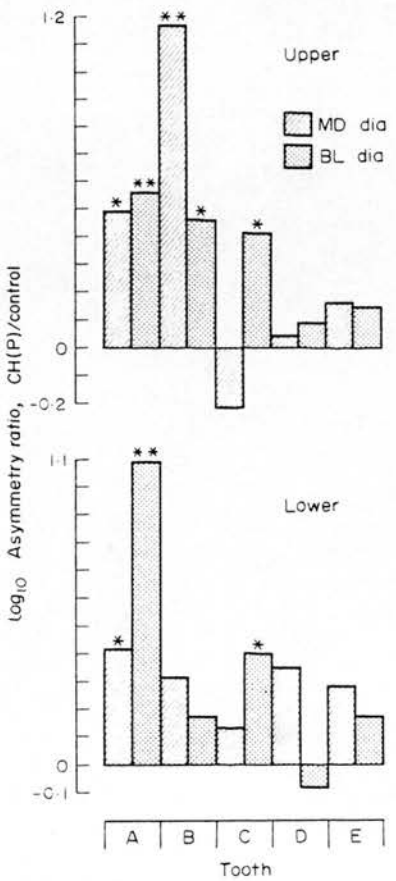


Fig. 2. The observed pattern of asymmetry in the deciduous dentition for all CL(P) cases relative to controls. For each tooth the log of the asymmetry ratio is shown for both mesiodistal and buccolingual diameters. Asterisks indicate that untransformed ratios differed significantly from unity (*$p < 0.05$; **$p < 0.01$).

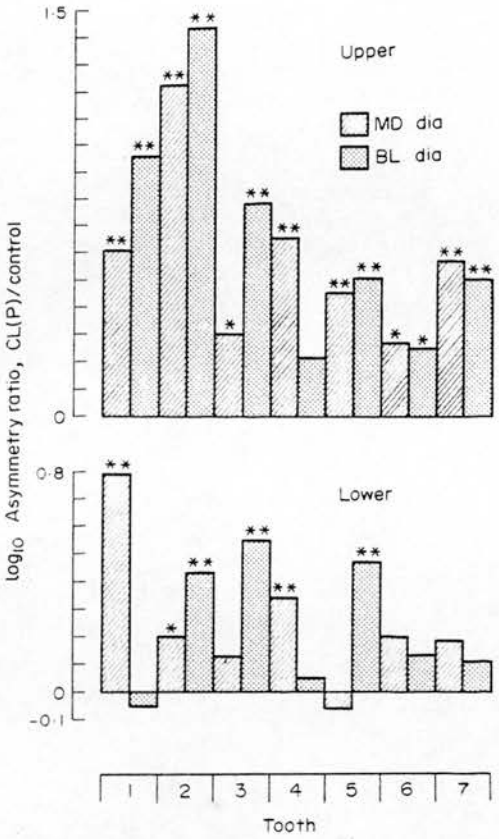


Fig. 3. The observed pattern of asymmetry in the permanent dentition for all CL(P) cases relative to controls.

groups should likewise occur throughout the lower jaw.

Figure 5 shows the comparison of FH + ve with FH − ve cases for the deciduous dentition. Taken as a whole there were two deciduous measurements showing significantly greater asymmetry, and two others showing significantly less asymmetry in the FH + ve group. These results are therefore inconclusive. However, the corresponding results for the permanent dentition (Fig. 6) are more in keeping with the expectations illustrated in Fig. 4c. Of the six significant differences found, five showed that the FH + ve group was more asymmetrical than the FH − ve group, and none of these differences occurred in the incisor region of the upper jaw.

DISCUSSION

In keeping with the pattern of asymmetry reported here, previous studies of dental abnormalities associated with CL(P) showed that, whereas the most extreme abnormalities of number, form and structure are almost always limited to the vicinty of the cleft, though some of these abnormalities may be the result of surgical intervention (Böhn, 1963; Dixon, 1968; Zilberman, 1973), less severe abnormalities either appear in other regions or are generalized throughout CL(P) dentitions. For example, Olin (1964) found that maxillary and mandibular premolars were absent more frequently in CL(P) cases than in the general population, whereas Jordan, Kraus and Neptune

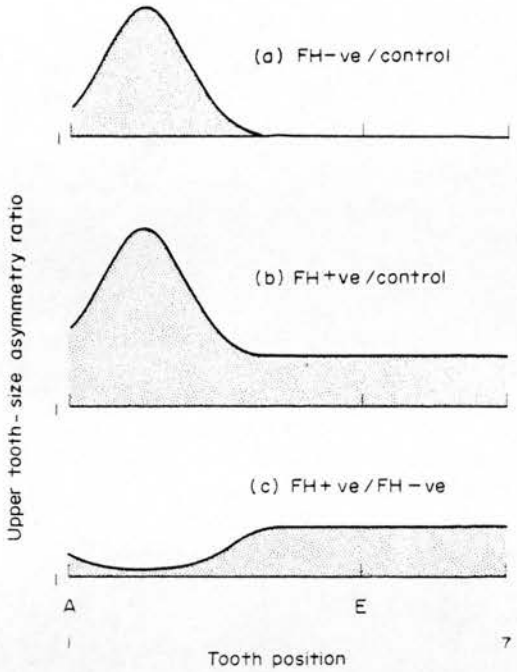


Fig. 4. Possible patterns for the asymmetry ratio in the upper jaw according to the 'reduced buffering' hypothesis.

(1966) observed that certain minor morphological abnormalities occurred in both the maxillary and mandibular teeth of cleft individuals, concluding reasonably that the abnormalities could not have been directly produced by the cleft. Furthermore, Foster and Lavelle (1971) found that a generalized reduction of tooth size in both the upper and lower jaws occurred with CL(P).

Ross and Johnston (1972) suggested that the widespread dental abnormalities associated with CL(P) are due to the deficiency of facial mesenchyme which plays a major part in the aetiology of primary palatal clefts, the deficient mesenchyme providing poor sup-

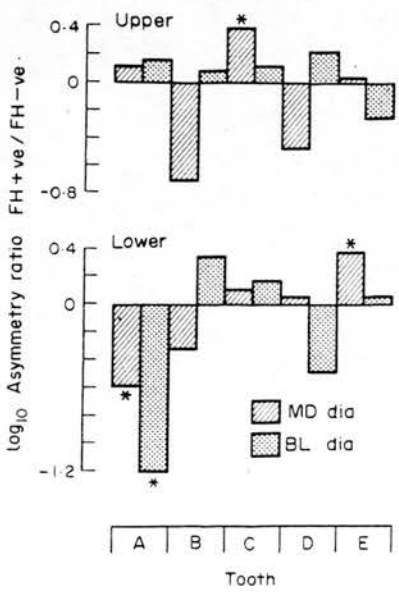


Fig. 5. The observed pattern of asymmetry in the deciduous dentition for FH + ve cases relative to FH − ve.

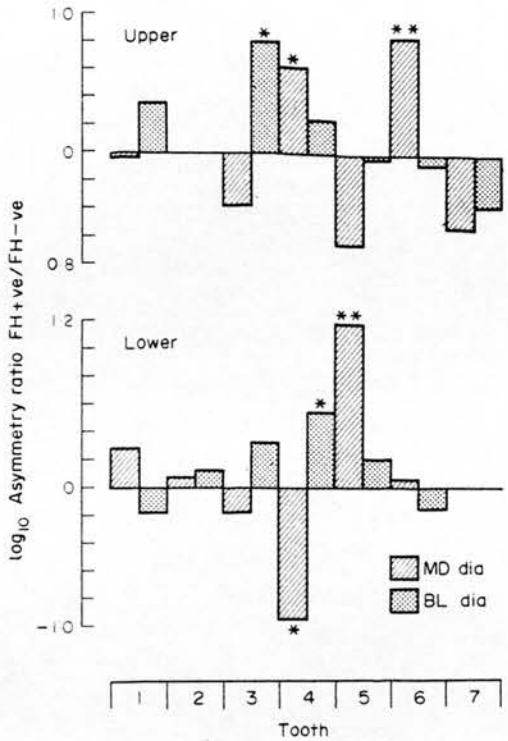


Fig. 6. The observed pattern of asymmetry in the permanent dentition for FH + ve cases relative to FH − ve.

port for the epithelial component of each developing tooth germ. An alternative explanation is that the feeding problems of CL(P) babies, together with the systemic effects of repeated surgical operations at an early age, may be responsible for generally abnormal or retarded dental development. Either of these explanations is consistent with unusually high levels of tooth-size asymmetry, because abnormal phenotypes, whether they have a genetic or an environmental basis, are more variable than normal. However, although these explanations are adequate from the purely dental point of view, the evidence for a difference between sporadic and familial CL(P), and the fact that dermatoglyphic asymmetry is also increased, not just in familial cases but in their non-cleft relatives also, means that another asymmetry inducing mechanism is in operation. The reduced buffering hypothesis of Adams and Niswander provides an attractive explanation of all the observations, though contributions to tooth-size asymmetry from mesenchymal deficiency, postnatal systemic effects and surgical intervention are not excluded. In fact, the greater asymmetry shown by the permanent dentition in CL(P) may be due to the superimposition of disrupting postnatal environmental effects on other influences that operate both prenatally and postnatally; the greater asymmetry shown by the permanent teeth of the familial as opposed to the sporadic group could be the result of greater sensitivity to these environmental effects.

Although the distinction between sporadic and familial groups is to some extent arbitrary, at least some of the sporadic cases presumably having a partly genetic aetiology; the difference between them could perhaps be of practical importance for counselling families in each of which there is, as yet, only one affected individual. Such families are presumably of two types: truly sporadic families, in which the malformation has a non-genetic aetiology and which, no matter how many subsequent offspring are produced, are likely to remain in the sporadic category; and genetically predisposed families, some of which will be transferred from the sporadic to the familial category by subsequent affected births.

Whatever the precise nature of the aetiological factors involved in producing CL(P), the sporadic and familial groups appear to be distinguishable one from the other on the basis of dental and dermatoglyphic asymmetry. Furthermore, non-cleft relatives of familial and sporadic cases also fall into separate groups, but as yet this distinction has been based only on dermatoglyphic asymmetry data. If, instead of distinguishing between large groups of individuals, it were possible to discriminate between truly sporadic and genetically-predisposed families on the basis of family asymmetry levels, more accurate information than at present available could be used for genetic counselling. A family shown by asymmetry studies to be of the truly sporadic type could be reassured that the chance of recurrence is negligible. However, in a family where abnormally high asymmetry levels point to a genetic predisposition, the risk of recurrence would be higher, perhaps considerably so, than the empirical risk of approximately 1:25 that applies to all small families containing one affected individual (Ross and Johnston, 1972; Gorlin, Pindborg and Cohen, 1976), the actual level of risk depending on the relative frequencies of truly sporadic and genetically-predisposed families.

Family asymmetry, to be sufficiently discriminating, would have to be expressed by a composite score, based on the asymmetry shown by several different bilaterally represented structures throughout the body. Dental measurements could make a substantial contribution to such a combined asymmetry score, and my study should provide helpful background information for any such work.

In conclusion, CL(P) individuals as a group showed a very clear pattern of asymmetry results. However, the comparisons between familial and sporadic cases, using smaller numbers of tooth pairs, were more equivocal, though for the permanent teeth the results were consistent with the reduced buffering hypothesis for the aetiology of CL(P). Similar investigations carried out on larger samples of CL(P) cases and their non-cleft relatives could perhaps provide additional worthwhile information. From the purely dental point of view, such studies could indicate whether or not the upper lateral incisor region in non-cleft relatives of familial cases shows signs of abnormally high developmental instability, even though no malformation has occurred. They could also provide information that might ultimately help to discriminate between a truly sporadic family and a genetically predisposed family in which there is, as yet, only one individual affected.

Acknowledgements—I am grateful to Dr. G. B. Hopkin, Head of the Orthodontic Department, Edinburgh Dental Hospital, for access to the casts of cleft and control cases; Mr. A. D. R. Batchelor, Consultant Plastic Surgeon, Royal

Hospital for Sick Children, for permission to study hospital records; and Miss Edith Redpath for making the measurements.

REFERENCES

Adams M. S. and Niswander J. D. 1967. Developmental "noise" and a congenital malformation. *Genet. Res.* **10,** 313–317.

Böhn A. 1963. Dental anomalies in harelip and cleft palate. *Acta odont. scand.* **21,** Suppl. 38.

Dixon D. A. 1968. Defects of structure and formation of the teeth in persons with cleft palate and the effect of reparative surgery on the dental tissues. *Oral Surg.* **25,** 435–446.

Foster T. D. and Lavelle C. L. B. 1971. The size of the dentition in complete cleft lip and palate. *Cleft Palate J.* **8,** 177–184.

Fraser F. C. 1970. Review: The genetics of cleft lip and palate. *Am. J. hum. Genet.* **22,** 336–352.

Gorlin R. J., Pindborg J. J. and Cohen M. M. 1976. *Syndromes of the Head and Neck.* 2nd Edn. McGraw-Hill, New York.

Jordan R. E., Kraus B. S. and Neptune C. M. 1966. Dental abnormalities associated with cleft lip and/or palate. *Cleft Palate J.* **3,** 22–55.

Olin W. H. 1964. Dental anomalies in cleft lip and palate patients. *Angle Orthodont.* **34,** 119–123.

Ross R. B. and Johnston M. C. 1972. *Cleft Lip and Palate.* Williams & Wilkins, Baltimore, MD.

Waddington C. H. 1957. *The Strategy of the Genes.* George Allen and Unwin, London.

Woolf C. M. and Gianas A. D. 1976. Congenital cleft lip and fluctuating dermatoglyphic asymmetry. *Am. J. hum. Genet.* **28,** 400–403.

Woolf C. M. and Gianas A. D. 1977. A study of fluctuating dermatoglyphic asymmetry in the sibs and parents of cleft lip propositi. *Am. J. hum. Genet.* **29,** 503–507.

Zilberman Y. 1973. Observations on the dentition and face in clefts of the alveolar process. *Cleft Palate J.* **10,** 230–238.

THE VERTEBRAL COLUMN AS A MODEL OF REGIONAL DIFFERENTIATION

Heterodont dentitions and the vertebral column are comparable in that each comprises a series of homologous structures divided into morphologically distinct classes. However, the vertebral column is composed of a much larger number of elements in each class, and this allows any morphological gradient to be sampled at a greater number of positions. The vertebral column is therefore a useful system in which to study the general problem of regional differentiation in a series of homologous structures. The effects of 24-hour starvation and of six different mutant genes on various measures of developmental stability in the mouse vertebral column indicate that the vertebral class boundaries are established at a very early stage of development, even before somite formation.

16. Morphogenetic Influences and Patterns of Developmental Stability in the Mouse Vertebral Column

J. A. SOFAER

School of Dental Surgery and Department of Human Genetics, University of Edinburgh, Scotland

Introduction

Developmental stability can be assessed through a study of the variation shown by adult populations. Two aspects of this variation are: firstly, the asymmetry of bilaterally represented structures within individuals; and secondly, in experimental situations, differences between members of a genetically homogeneous population. Asymmetry provides information about the ability of the individual to produce the same developmental result on two occasions, and is perhaps the most controlled indication of instability of development, since, under normal circumstances, the genotype and general external environment are the same for both sides of the body. Between-individual variation in a genetically homogeneous population expresses the lack of ability of a given genotype to produce the same developmental result on several occasions, though it must be assumed that all individuals are in fact genetically identical and that the general environment is constant. If different organs or regions of the body are studied with respect to asymmetry and between-individual differences, the resulting patterns of variation can be regarded as patterns of relative developmental instability, and can, perhaps, throw some light on the distribution of morphogenetic influences that may be operating during development. A further dimension can be added to such an investigation by examining the response of these patterns to a standard environmental disturbance imposed at different stages of development.

A Suitable System

In a series of homologous structures divided into morphologically distinct

segments, such as the teeth of most mammals, differences within and between segments with respect to asymmetry or the response to an imposed environmental disturbance can provide patterns of instability that may suggest how these morphologically distinct regions are established during development. However, the dentition of the most convenient experimental mammal, the mouse, is far from ideal for such a study. There are only two morphological classes of teeth, incisors and molars, represented by only one and three teeth respectively in each quadrant. A series of homologous structures more suitable for the study of patterns of developmental stability is found in the mouse vertebral column. Anterior to the sacrum there are three morphological segments: cervical, thoracic and lumbar, normally composed of 7, 13 and 5 or 6 vertebrae respectively. The potential for disclosing patterns of morphogenetic influence is much greater among these 25 or 26 presacral vertebrae than among the smaller number of teeth, since the effect of any such influence can, as it were, be "sampled" at a greater number of positions.

The vertebrae, of course, are not bilaterally represented structures, so asymmetry cannot be studied in the same way as for teeth; but each vertebra has a right and a left half that can be measured and compared. It was therefore decided to investigate vertebral asymmetry, and the response of vertebral size and asymmetry to an environmental disturbance, in an inbred strain of mice; the aim being to assess the value of the mouse vertebral column for studying the way in which morphological segments in a series of homologous structures arise during development.

Embryological Background

Before proceeding to the experiment itself, it is relevant to consider briefly the early embryology of vertebral development.

Figure 1 is a summary of early mouse development. If the day on which fertilization occurs is regarded as day 0, then implantation occurs between days 4 and 5, mesoderm starts to appear between days 6 and 7, and somite development starts on day 8. The first five somites contribute to the occipital region of the skull, and the vertebrae are derived from subsequent somites. Each vertebra is formed from the neighbouring caudal and cranial halves of two adjacent somites, so there is a one-to-one somite–vertebra correspondence, though the sequence of vertebrae is displaced by half a unit relative to that of the somites. The somites from which the presacral vertebrae are formed have all appeared by the end of day 10 (Grüneberg, 1963; Snell and Stevens, 1966; Theiler, 1972). It is during the time when their somites are forming that the vertebrae have been found to be most sensitive to teratogenic treatments. Variations in both vertebral number and form have been produced by a variety of such treatments, including fasting, hypoxia, X-radiation and a number of different chemical agents (Dagg, 1966).

Material and Methods

The material consisted of a control group and six groups of mice that were

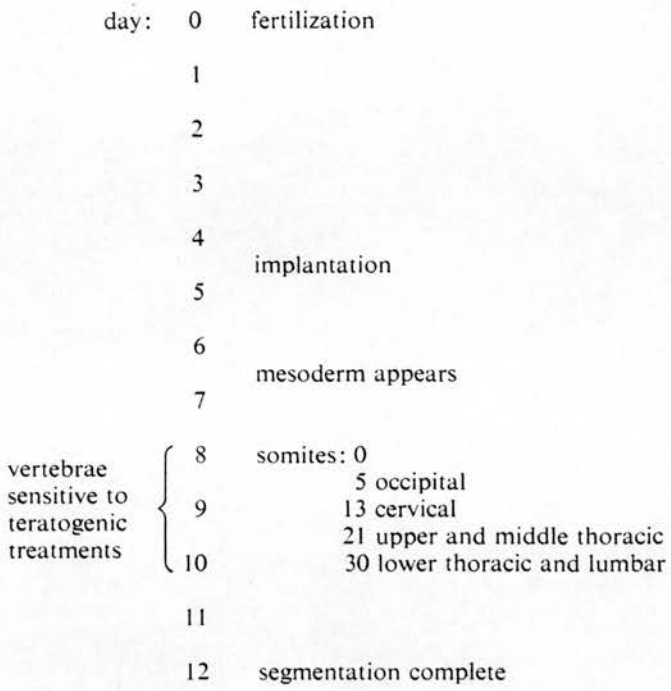


FIG. 1. A summary of early mouse development.

the progeny of females starved for 24 h on one of six different days of pregnancy. All mice were from the highly inbred strain CBA/Cam, so for practical purposes they could be considered genetically identical.

Females were caged with males (three females to one male per cage) and were examined for vaginal plugs on the following morning. Plugged females were then caged individually with *ad libitum* supplies of food and water. The day on which a plug was found was regarded as day 0. Females for starvation were transferred to individual clean cages without food, but with water as before, at 16·00 h on day $n-1$ and returned to their original cages supplied with food and water at 16·00 h on day n, where n was one of the following: 5, 6, 7, 8, 10 or 12. Litters from starved and control mothers, if larger than six, were reduced to six as soon as possible after birth. All offspring were weaned at 4 weeks, stored six to a cage, and sacrificed at 6 weeks after birth. The numbers of mice used and the numbers of litters from which they came are shown in Table I. Within each group there were approximately equal numbers of the two sexes.

After sacrifice, each vertebral column down to the sacrum was dissected out with its immediately adjacent tissues, and a hard stainless steel wire (0·25 mm diameter) was threaded down the neural canal and twisted at each end. The column was then subjected to papain digestion, normally formed and unbroken vertebrae remaining in the correct order on the wire. A search

TABLE I. The numbers of mice used and the numbers of litters from which they came.

Starvation day	Individuals	Litters
5	50	12
6	35	6
7	25	7
8	29	6
10	50	12
12	50	10
Control	39	8

was made following papain digestion for any abnormally formed or broken vertebrae that may have fallen from the wire.

Measurement Technique

Vertebrae from each column were attached, cranial surface uppermost, onto a 3·25 inch square lantern slide glass by means of double-sided transparent self-adhesive tape, and silhouettes of each complete set of presacral vertebrae, plus the first sacral vertebra, were projected onto a screen at a convenient standard magnification (×17·5). Figure 2 illustrates the kind of picture that was produced. Vertebra no. 1 is the first cervical, and vertebra no. 26 is the first sacral. The first sacral vertebra was included because, as it articulates with the pelvis, it was thought likely to be a relatively stable structure with which the presacral vertebrae could be compared. Even though the vertebrae vary considerably, each one, except the first, has a dorsal mid-line spinous process, and all have lateral transverse processes; though in some vertebrae they are poorly developed. The first cervical vertebra has a mid-line ventral tubercle.

Two measurements were taken from each of these vertebral silhouettes by placing a grid of millimetre squared graph paper against the screen and adjusting its position until the transverse processes of each silhouette came to lie along one axis. The two measurements taken were the distances along this axis from the end of each transverse process to a perpendicular line passing through the spinous process, or, in the case of the first cervical vertebra, the centre of the ventral tubercle. This is illustrated in Fig. 3.

In the few cases where the complement of presacral vertebrae differed from 25, adjustments in numbering were made so that the first sacral vertebra always occupied position 26 in the series. When there was an additional vertebra, one was removed from the series, and when there was one less than the normal 25, a space was left in the appropriate position in the series. This was done so that sets of measurements in such cases would be comparable with those of the majority of individuals with the normal vertebral number.

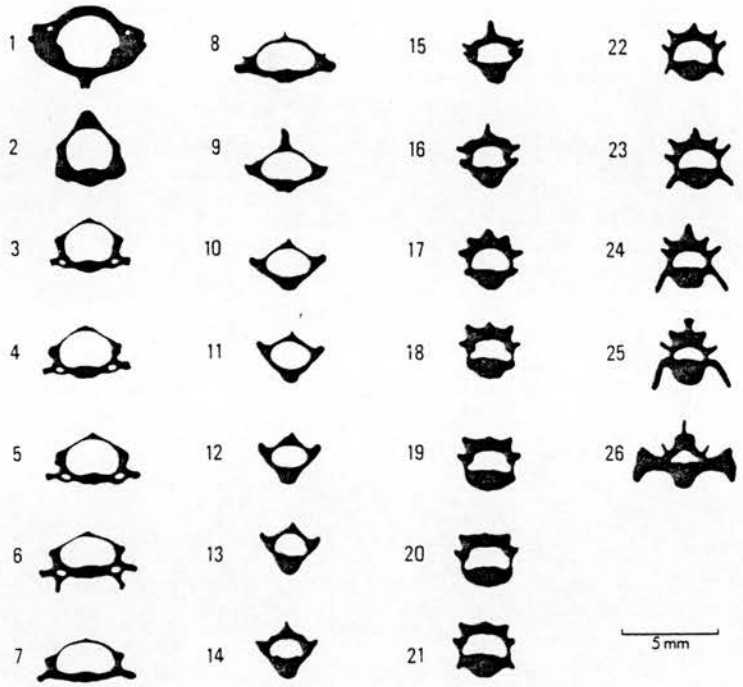


FIG. 2. Silhouettes from a control vertebral column.

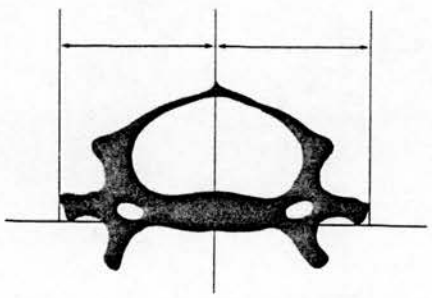


FIG. 3. Silhouette of vertebra no. 6 showing the measurements that were made.

Measures of Developmental Instability

Asymmetry was expressed by V_a, the variance of $(R - L)/(R + L)$, where R and L are corresponding measurements on the right and left sides of each vertebra. This measure of asymmetry is therefore adjusted for the size of the particular vertebra being considered. The response to starvation was expressed firstly by relative asymmetry, the ratio of the experimental asymmetry variance to the control asymmetry variance for each vertebra and for each

starvation group; and secondly by the percentage change of full vertebral width (the sum of right and left side measurements) associated with starvation at each stage.

Since the anatomical landmarks used to provide reference points for measurement are not equally well defined in all vertebrae, different degrees of measurement error could arise for different vertebrae. In order to minimize the possibility of such differences masking or distorting any underlying pattern of instability, the control group of vertebrae was measured on a second occasion. A repeatability variance, V_r, for each vertebra, comparable with the asymmetry variance, could then be calculated as the variance of $(1st - 2nd)/(1st + 2nd)$ measurements, for the right and left sides separately. Right and left side repeatability variances were combined to give a single overall estimate of the repeatability variance for each vertebra, and two asymmetry variances, one calculated from the first and the other from the second set of measurements, were similarly combined to give a single overall estimate of the asymmetry variance for each vertebra. The "instability variance", V_i, of the control group was considered to be the difference between the asymmetry and repeatability variances for each vertebra. Thus, for each vertebra,

$$V_i = V_a - V_r.$$

Patterns of developmental instability were expressed graphically, with the measure of instability (control instability variance, relative asymmetry or % change of width) plotted against vertebral position number. Positions 1–7 refer to the cervical segment, 8–10 the thoracic segment, 21–25 the lumbar segment and 26 the first sacral vertebra. All measures of instability were based on vertebral measurements expressed in mm.

Results

The numbers of presacral vertebrae that were found in the different groups are shown in Table II. The halves arise because sometimes there is asymmetrical articulation with the pelvis (McLaren and Michie, 1954). However, for the purpose of standardizing the recording of measurements, cases falling

Table II. The numbers of presacral vertebrae found in the different groups.

Starvation day	Vertebrae 24	24½	25	25½	26
5	–	–	50	–	–
6	–	–	35	–	–
7	–	–	25	–	–
8	2	–	23	3	1
10	–	–	49	1	–
12	–	–	50	–	–
Control	–	–	39	–	–

into the 25½ category were regarded as having 26 presacral vertebrae, so in these cases one complete lumbar vertebra was removed from the series. Exceptions to the normal presacral number of 25 in this strain occurred only in the groups starved on days 8 and 10.

Figure 4 shows the pattern of instability variances in the control group for

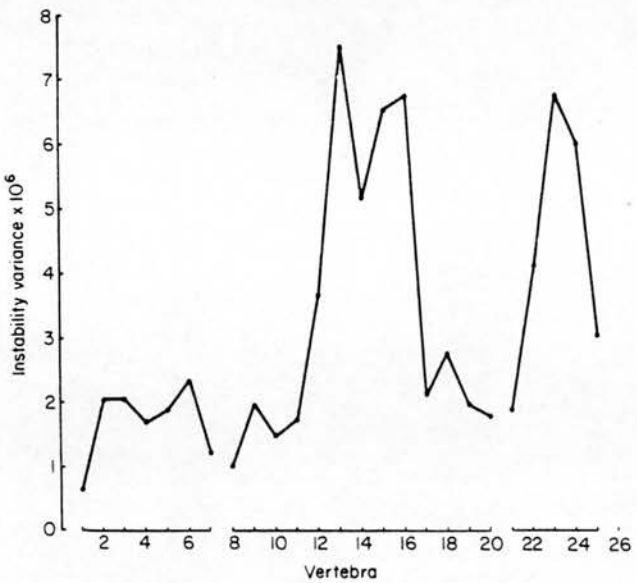


FIG. 4. The pattern of instability variances in the control group.

the 26 vertebrae that were measured. The most striking feature is that vertebrae towards the extremes of each segment were the least unstable, and those towards the centre of each segment were the most unstable. However, at the very centre of the cervical and thoracic segments there was evidence of a small localized decrease of instability.

The relative asymmetry for the different groups, that is, the variance ratio of the experimental asymmetry variance to the control asymmetry variance, is shown in Fig. 5. There was little by way of a consistent response to starvation on days 5, 6 and 7, though, at least in the cervical segment, vertebrae towards the centre tended to be more affected by starvation than those at the extremes. However, there was a definite response to starvation on day 8 in the thoracic segment. Vertebrae at the extremes of the segment tended to be least affected by starvation, whereas those towards the centre showed a marked increase of asymmetry, with a localized decrease of the starvation effect a little anterior to the very centre of the segment. Starvation on days 10 and 12 tended to increase asymmetry on the whole, though there was no very definite pattern of response.

The response of the full width of each vertebra to starvation is shown in

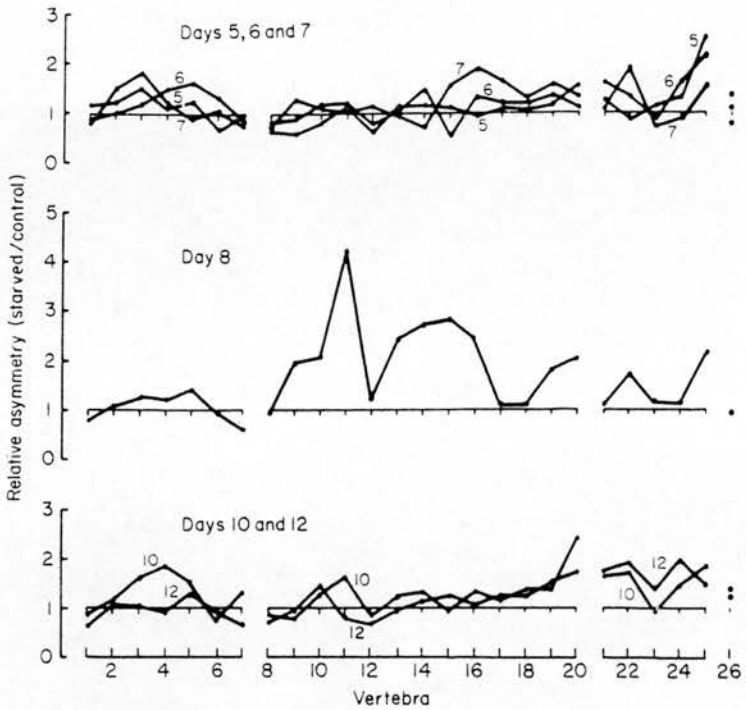


FIG. 5. Patterns of relative asymmetry following starvation on different days of pregnancy.

Fig. 6. Surprisingly, the general effect of starvation on days 5 and 6 was to increase vertebral width. However, by day 8 the effect of starvation was definitely to reduce vertebral width, and this applied also to starvation on days 10 and 12. For days 5 and 6 the cervical and thoracic segments again tended to show least response at their extremes and most response towards their centres, with some suggestion of localized resistance to the starvation effect at the very centre of each segment. By day 8 the pattern became one of relative stability at the anterior end and instability at the posterior end of each segment, particularly of the thoracic segment. A similar pattern in the thoracic segment was found for day 10, with some relaxation of the effect by day 12.

DISCUSSION

Theoretical Considerations

Three different patterns of developmental instability that might result from different kinds of morphogenetic control are illustrated in Fig. 7. In the first scheme, against (a), the morphogenetic influence for each segment, cervical (C), thoracic (T) and lumbar (L), has its origin at the anterior end of each segment and spreads posteriorly. Against (b) is the associated pattern of instability that might be expected. Vertebrae at the anterior end of each

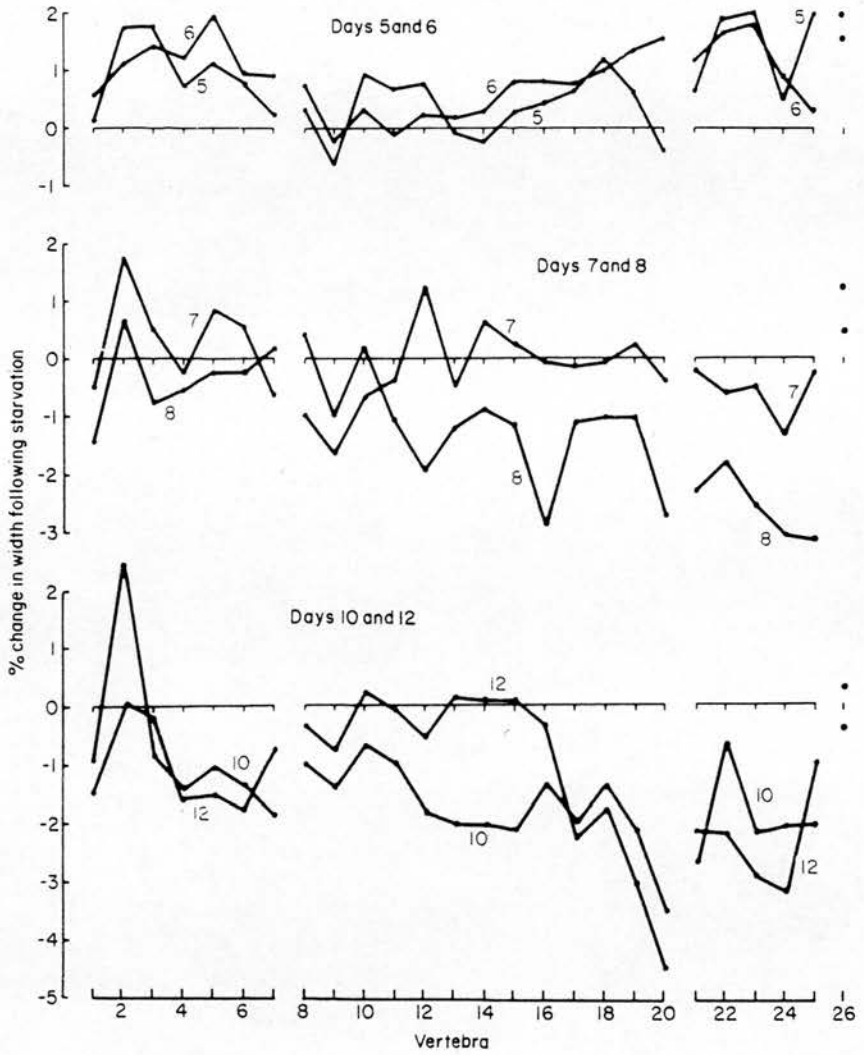


FIG. 6. Patterns of percentage change in width following starvation on different days of pregnancy.

segment, closest to the source of morphogenetic influence, show least instability, whereas those at the posterior end, furthest away from the morphogenetic source, are most unstable. In the second scheme, the morphogenetic influence has its origin at the centre of each segment and spreads outwards. The corresponding pattern of instability shows least instability at the centre of each segment and greatest instability at their extremes. The third scheme shows the sources of morphogenetic influence defining the

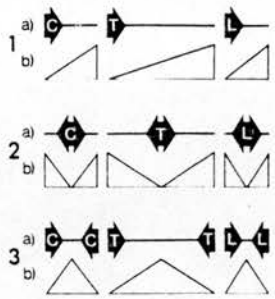


FIG. 7. Possible alternative schemes of morphogenetic control.

limits of each segment and spreading towards their centres. The corresponding pattern of instability is characterized by least instability at the ends and greatest instability at the centre of each segment.

Observed Patterns of Instability

The most common pattern of instability observed within segments was one of least instability towards the extremes and greatest instability towards the centre, corresponding to morphogenetic scheme no. 3 in Fig. 7. This pattern was found in all three segments for the control instability variance, in the cervical and thoracic segments of some of the starvation groups for relative asymmetry, and in the cervical segment for percentage change in full width. Superimposed on this pattern was some evidence of morphogenetic scheme no. 2, that is, a localized tendency towards a low level of instability at or near the very centre of a segment. This appeared in the cervical and thoracic segments for the control instability variance, in the thoracic segment for relative asymmetry on day 8, and also possibly in the lumbar segment for relative asymmetry. There was also some suggestion of morphogenetic scheme no. 1, particularly in the thoracic segment for percentage change in full vertebral width after starvation on days 8, 10 and 12. The first sacral vertebra was relatively stable in almost all situations.

The apparent increase in vertebral size following starvation on days 5 and 6 could perhaps have been due to physiological over-compensation by the pregnant female on her return to a cage supplied with food. Such over-compensation would have coincided with the time when the developing vertebrae were most sensitive to environmental effects. Even though this response was in the opposite direction to that produced by starvation at later stages, the pattern of low instability (resistance to size increase) towards the ends, and high instability (maximum size increase) towards the centre of the cervical segment, with a localized tendency towards less instability at the very centre, still applied.

Components of Asymmetry

The expression of asymmetry used here was the total observed asymmetry,

without regard to details of its underlying basis. However, the observed differences between sides can be thought of as having three components: fluctuating asymmetry, directional asymmetry and antisymmetry (van Valen, 1961). Fluctuating asymmetry is due to inability to buffer against minor developmental accidents and non-specific local environmental differences between sides, the distribution of $R - L$ being normal and having a mean of 0. Directional asymmetry is due to a consistently greater degree of development of the organ on one particular side of the body, presumably due to a consistent and "normal" difference in the local environment of the developing organ between sides. Here the distribution of $R - L$ is probably approximately normal, but the mean is different from 0. Antisymmetry occurs when a difference between sides is regularly induced by negative, presumably competitive, interaction between sides, $R - L$ having a mean of 0, but not being normally distributed, since individuals with a near-zero difference between sides are relatively rare. All three components arise through a lack of rigid intrinsic control by the organ over its own development, so an overall expression of asymmetry is a reasonable indication of developmental instability in its broadest sense. However, a difficulty arises if the directional and antisymmetry components take up more than a small proportion of the total observed asymmetry, for the observed distribution of $R - L$ would then deviate from normality. In this event, the variance of the difference between sides would not be a strictly valid measure. However, the general consistency of the patterns of instability obtained from the different measures in the different segments of the vertebral column suggests that this difficulty has not given rise to undue error in the present case. Nevertheless, it would perhaps be informative to look more deeply into the developmental basis of vertebral asymmetry and vertebral size variation as a whole, particularly with regard to possible interactions between sides within vertebrae, and interactions between adjacent vertebrae.

Implications of the Results

The results therefore suggest that the predominant morphogenetic influence in the establishment of the segments in the vertebral column is one in which a prominent feature is the defining of the limits of each segment. There is evidence that superimposed on this is an influence originating at the centre of the cervical and thoracic segments; and there is also evidence for an effect that originates at the anterior end of the thoracic segment, spreading posteriorly. All effects related to starvation showed a maximum with starvation on day 8, which is consistent with previous teratological experiments. The segmental response of asymmetry and vertebral width to starvation indicates that division of the vertebral column into prospective morphological segments is already established at the stage of somite development. The thoracic segment, the longest segment, showed the greatest range of effect for all measures of instability, which tends to support the concept of morphogenetic influences spreading outwards from well defined sources, their effects weakening with distance.

An analysis of truly morphological, rather than size, variation over the length of the vertebral column could perhaps serve to confirm these findings and be more specific about the way in which vertebral development is controlled. The value of a detailed morphological analysis of the vertebral column, in terms of underlying morphogenetic gradients, has already been suggested (van Valen, 1970). The possibility of investigating vertebral development through direct experimental manipulation in embryos of oviparous amniotes has also been suggested (van Valen, 1970), and, more recently, manipulations of this type have been performed on chick embryos with a view to investigating regional determination in the vertebral column (Kieny *et al.*, 1972). In these experiments, segmented or even unsegmented somitic or presomite mesoderm from the cervical region of a donor embryo was transferred to the thoracic region of a host embryo from which a corresponding piece of mesoderm had been removed. A similar transfer of segmented or unsegmented thoracic mesoderm was made to the cervical region. In both types of transfer the transplanted mesoderm developed according to its origin, resulting either in a rib-free region within the thoracic segment, or supernumerary ribs in the cervical segment. The fact that unsegmented mesoderm behaved in this way indicates that, in the chick, the somitic mesoderm has acquired regional determination before segmentation occurs.

Summary

Patterns of developmental instability in the mouse vertebral column have been studied with a view to drawing general conclusions about influences that divide a series of homologous structures into morphologically distinct segments. It is suggested that the results indicate patterns of morphogenetic control that might be less easily disclosed in dentitions, where the number of elements in each segment is smaller. In addition, the possibilities for more detailed investigation discussed, particularly direct experimental interference at an early embryonic stage in the chick, make the vertebral column a useful system for studying the way in which morphological segments in a series of homologous structures arise during development.

Acknowledgements

The material was collected at the University of Cambridge, Department of Genetics, and for this thanks are due to Professor J. M. Thoday for laboratory facilities, the Nuffield Foundation for a Dental Research Fellowship and the Medical Research Council for a Research Project Grant. Edith Redpath made the measurements in Edinburgh.

References

Dagg, C. P. (1966). Teratogenesis. *In* "Biology of the Laboratory Mouse" (Green, E. L., ed.), Ch. 14. McGraw-Hill, New York, Toronto, Sydney and London.

GRÜNEBERG, H. (1963). "The Pathology of Development". Blackwell, Oxford.

KIENY, M., MAUGER, A. and SENGEL, P. (1972). Early regionalisation of the somitic mesoderm as studied by the development of the axial skeleton of the chick embryo. *Devl Biol.* **28**, 142–161.

MCLAREN, A. and MICHIE, D. (1954). Factors affecting vertebral variation in mice. I. Variation within an inbred strain. *J. Embryol. exp. Morph.* **2**, 149–160.

SNELL, G. D. and STEVENS, L. C. (1966). Early embryology. *In* "Biology of the Laboratory Mouse" (Green, E. L., ed.), Ch. 12. McGraw-Hill, New York, Toronto, Sydney and London.

THEILER, K. (1972). "The House Mouse. Development and normal stages from fertilisation to 4 weeks of age." Springer-Verlag, Berlin, Heidelberg and New York.

VAN VALEN, L. (1961). A study of fluctuating asymmetry. *Evolution* **16**, 125–142.

VAN VALEN, L. (1970). An analysis of developmental fields. *Devl Biol.* **23**, 456–477.

GENETIC AND ENVIRONMENTAL INFLUENCES ON PATTERNS OF DEVELOPMENTAL STABILITY IN THE MOUSE VERTEBRAL COLUMN

J.A. Sofaer

Running title:

Developmental stability in the mouse vertebral column

Key words: Mouse, Vertebrae, Developmental stability

University of Edinburgh,
Department of Oral Medicine and Oral Pathology,
Old Surgeons Hall, High School Yards,
Edinburgh EH1 1NR

and

University Department of Human Genetics,
Western General Hospital,
Edinburgh EH4 2XU

SUMMARY

The effects of six mutant genes (Sd, T, vt, Sp, un, Ph), which influence different aspects of development of the axial skeleton in the mouse, were studied both alone and in combination with 24-hour starvation on day 8 of gestation, when teratogenic agents are known to have a pronounced influence on this system. Responses to the mutant genes were assessed in terms of mean size, size variation and asymmetry of the presacral and first sacral vertebrae of young adult mice. Normal controls showed craniocaudal morphological gradients and relatively low variability towards the boundaries of the cervical, thoracic and lumbar vertebral classes. Gene-induced craniocaudal gradients of abnormality over the series were observed in response to Sd and T, and to Ph, which have direct and indirect effects respectively on the primitive streak and/or notochord. By contrast, vt, which influences the unsegmented paraxial mesoderm, and Sp and un, which act later, during segmentation and sclerotome differentiation respectively, did not produce overall craniocaudal gradients of abnormality. However, all six genes revealed a pattern of relative resistance to their effects towards the vertebral class boundaries. The division of the axial skeleton of the mouse into its morphologically distinct classes of vertebrae thus appears to originate at the earliest stage of axial development, even before the onset of somite formation, with the positions of the future vertebral class boundaries established both axially and paraxially as regions of relative stability that persist into later stages of development. The primary craniocaudal gradient associated with the advancing primitive streak, from which the vertebral class boundary positions may be derived, appears to be restricted to the axial structures themselves and to be lost after the onset of segmentation.

INTRODUCTION

There has long been speculation as to how a series of homologous structures becomes differentiated into morphologically distinct classes during development. A clear example occurs in heterodont dentitions where early rudiments of the teeth of all classes (e.g. incisor, premolar, molar) appear to be indistinguishable, and yet considerable differences between classes become apparent as development proceeds. A comparable situation is found in the axial skeleton, where apparently similar somites contribute to a number of distinct vertebral classes (e.g. cervical, thoracic, lumbar). Various theoretical explanations have been formulated and, as Lumsden (1979) has pointed out in relation to dentitions, these fall into two categories: 'gradient' theories, where all primordia are initially equivalent and differentiate in response to the gradient of a morphogen (or morphogens) extrinsic to the primordia (Butler, 1967; Van Valen, 1970); and cell lineage theories, where the fate of each primordium is determined by its own developmental history (Osborn, 1978). Within the mouse molar class, cell lineage seems to be responsible for the observed morphological gradient (Lumsden, 1979). However, at some time before the operation of such a class as an autonomous unit, the boundaries between classes (or prospective classes) must be defined.

The purpose of the investigation reported here was to try and disclose possible influences leading to the establishment of morphologically distinct classes in a series of homologous structures. The system chosen was the mouse vertebral column in which, cranial to the sacrum, there are three such classes: cervical, thoracic and lumbar, normally composed of 7, 13 and 5 or 6 vertebrae respectively.

Compared with dentitions, the relatively large number of elements in each class allows any morphological gradient to be sampled at a greater number of positions. A previous study has already demonstrated relative stability of the vertebral class boundary regions in normal inbred mice and, by means of 24-hour maternal starvation at different stages of pregnancy, has suggested that this relative stability may be established early in vertebral development (Sofaer, 1978). The present paper is concerned with the effects of six mutant genes, known to influence different aspects of development of the axial skeleton, both alone and in combination with 24-hour starvation on day 8 of gestation. It is around this stage of gestation that the vertebrae have been shown to be most sensitive to a variety of teratogenic agents (Dagg, 1966). It was anticipated that the mutant genes, with and without the accompanying environmental stress, would result in a wide range of disruption of the normal pattern of developmental stability in the axial skeleton, and that a consideration of the abnormal patterns produced, in the light of what is known of the site and time of action of each of the genes, might shed more light on how and when the morphological classes in the mouse vertebral column are established during development.

MATERIAL AND METHODS

Genes and genetic background

The six genes and their primary effects are listed in Table 1. Sd and T were obtained from the Institute of Animal Genetics, Edinburgh, and vt, Sp, un and Ph from the MRC Radiobiology Unit, Harwell.

For the dominant genes, mothers of the mice studied were all inbred females from the strain CBA/ca (University of Edinburgh, Centre for Laboratory Animals) and fathers were heterozygotes resulting from 1 to 3 crosses of the original mutant stock to CBA/ca. Mice studied therefore comprised mutants (heterozygotes) and control (normal homozygote) littermates with a largely CBA/ca genetic background. For the recessive genes, mothers were the heterozygous offspring of crosses between CBA/ca and the original mutant stock and fathers were homozygotes from the mutant stock. Mice studied therefore comprised mutants (homozygotes) and control (heterozygote) littermates with a partially CBA/ca genetic background.

Specimens from different genotype/environment groups

Females chosen to be mothers were caged with the appropriate males, three females to one male per cage. Females to be starved during pregnancy were examined for vaginal plugs each morning, the day on which a plug was found being regarded as day 0. Females for starvation were transferred to individual clean cages without food, but with water, at 16.00 h on day 7 and food was resupplied from 16.00 h on day 8. Females exposed to a normal environment during

pregnancy were transferred to individual cages when obviously pregnant, being supplied with both food and water continuously. All offspring were weaned at 4 weeks, stored 6 to a cage and sacrificed at 6 weeks after birth.

After sacrifice, each vertebral column down to the sacrum was dissected out with its immediately adjacent tissues, and a hard stainless steel wire (0.25 mm diameter) was threaded down the neural canal and twisted at each end. The column was then subjected to papain digestion (Luther, 1949), normally formed and unbroken vertebrae remaining in the correct sequence on the wire. A search was made following papain digestion for any abnormally formed or broken vertebrae that may have fallen from the wire.

For each of the genes, specimens of vertebral columns were collected from mutants and their control littermates that had either undergone normal gestation or whose mothers were subjected to 24-hour starvation on day 8 of gestation. For each of the six genes there were thus four genotype/environment groups, making a total of 24 groups in all. It was the intention to collect 50 mice in each group, but some of the numbers fell short of this because of poor fertility. Eighteen of the groups comprised the full 50 mice each, five groups comprised 37-49 mice and one comprised 26 mice. Within the groups the sexes were represented in approximately equal proportions.

Measurement

Vertebrae from each column were attached, cranial surface uppermost, onto a 3.25 inch square lantern slide glass by means of double-sided transparent self-adhesive tape, and silhouettes of each

complete set of presacral vertebrae, plus the first sacral vertebra, were projected onto a screen at a standard magnification (x 20). The first sacral vertebra was included because, as it articulates with the pelvis, it was thought likely to be a relatively stable structure with which the presacral vertebrae could be compared. Even though the vertebrae vary considerably in size and morphology, each one, except the first cervical, has a dorsal midline spinous process, and all have lateral transverse processes; though in some vertebrae they are poorly developed. The first cervical vertebra has a midline ventral tubercle.

Two measurements were taken from each of these vertebral silhouettes by placing a grid of millimetre squared paper against the screen and adjusting its position until the transverse processes of each silhouette came to lie along one axis. The two measurements taken were the distances along this axis from the end of each transverse process to a perpendicular line passing through the spinous process, or, in the case of the first cervical vertebra, the centre of the ventral tubercle. This is illustrated in Fig. 1.

In cases where the complement of presacral vertebrae exceeded the most usual number of 25, an adjustment in numbering was made by removing one (or more) of the lumbar vertebrae. (In only one mouse were there more than 26 presacral vertebrae.) In this way all sets of measurements were comparable, each relating to a series of 26 vertebrae numbered in a craniocaudal sequence, with position 26 always occupied by the first sacral vertebra.

Since the anatomical landmarks used to provide reference points for measurement are not equally well defined in all vertebrae, different degrees of measurement error could arise for different vertebrae. In

order to minimise the possibility of such differences masking or distorting any underlying pattern of instability all vertebrae were set up on the glass slides and measured on two separate occasions. For each vertebra in each individual there were therefore first and second measurements on the right side, R_1 and R_2, and first and second measurements on the left side, L_1 and L_2. The width of each vertebra in each individual was expressed in terms of the average for each pair of duplicate measurements: $R = (R_1 + R_2)/2$ and $L = (L_1 + L_2)/2$. From these R and L values the following parameters were calculated for each group:

1) Mean and standard error of $(R + L)$, expressing overall vertebral width;
2) Coefficient of variation of $(R + L)$, expressing between individual variation for width, independent of vertebral size;
3) Mean and standard error of $(R - L)/(R + L)$, expressing directional asymmetry, independent of vertebral size.

In addition, for each vertebra in each group, asymmetry was expressed as the variance of $(R_1 - L_1)/(R_1 + L_1)$ and of $(R_2 - L_2)/(R_2 + L_2)$ for the first and second sets of measurements, and repeatability was expressed in terms of the variance of $(R_1 - R_2)/(R_1 + R_2)$ and of $(L_1 - L_2)/(L_1 + L_2)$ for the right and left sides respectively. A fourth parameter for each group, the 'instability' variance, V_I, was then calculated as $V_I = V_A - V_R$ where V_A was the pooled asymmetry variance for first and second sets of measurements, and V_R the pooled repeatability variance for the right and left sides. The instability variance was thus independent of both vertebral size and repeatability.

Comparisons between groups were then made in terms of:

1) Per cent difference in mean width;

2) Width variance ratio;

3) Instability variance ratio;

4) Difference in mean directional asymmetry.

The relative magnitude of the differences between test and control groups at different positions in the vertebral column for each of these comparisons was taken to be an indication of developmental instability. Patterns of developmental instability were expressed graphically, with each measure of instability plotted against vertebral position number. Positions 1-7 refer to the cervical class, 8-20 the thoracic class, 21-25 the lumbar class and 26 the first sacral vertebra.

RESULTS

Vertebral number

The numbers of mice with 25, 25½ or 26 presacral vertebrae in the different genotype environment groups are shown in Table 2. Table 3 shows that in cases of asymmetrical articulation with the pelvis, the more caudal articulation was found more frequently on the right side.

Baseline patterns of variation for wildtype mice in the normal environment

Baseline patterns of variation for wildtype mice were obtained by pooling the results from control littermates of the four dominant mutants in the normal unstarved environment. Figure 2 illustrates patterns for the mean and coefficient of variation of vertebral width, and for the instability variance.

Mean vertebral width ranged from over 5 mm for the first cervical vertebra to just below 3 mm in the lower thoracic and upper lumbar region. Apart from the first cervical vertebra, which is a special case because of its articulation with the base of the skull, each class showed its own size gradient, with a change of gradient direction at or near the cervicothoracic (C-T) and thoracolumbar (T-L) class boundaries. Male vertebrae were consistently larger than female vertebrae, but generally by only 1-2 per cent. For the smaller genotype/environment groups, results for mean vertebral width are therefore given for both sexes combined.

There was an overall trend for the coefficient of variation of vertebral width to increase in a craniocaudal direction. However, superimposed on this trend were clear indications of resistance to this increasing variability towards the C-T and T-L boundaries. Males showed generally higher values over the whole series, with indications of minimum sexual dimorphism towards the class boundaries and in the mid-thoracic region. For the smaller genotype/environment groups, results are given for both sexes combined.

The instability variance showed no consistent difference between the sexes and so only pooled values are illustrated. The pattern was one of minimum instability towards the vertebral class boundaries, except at the caudal end of the lumbar class.

Figure 3 illustrates the pattern of directional asymmetry where again there was no consistent difference between the sexes. There were two peaks of left-sided predominance, one primarily cervical and the other thoracic, and one primarily lumbar peak of right-sided predominance.

Response to 24-hour starvation on day 8 of gestation

The effects of starvation alone were assessed by comparing wildtype control progeny of starved mothers with wildtype control progeny of unstarved mothers for the four dominant mutant stocks combined.

Starvation produced a modest increase in mean vertebral width (1-5%) over the whole series of 26 vertebrae. This increase in size was statistically highly significant ($p < 0.01$) for 24 of the 26 vertebrae, and there was no obvious difference of effect in the class boundary regions. The variance of vertebral width tended to be

reduced by starvation. The instability variance showed a tendency for an overall increase of instability with starvation, but with more normal levels towards the cranial and caudal ends of the series. Starvation tended to exaggerate the directional asymmetry shown by control mice in the normal environment, but this difference between environments only reached statistical significance ($p < 0.05$) for two vertebrae in the lumbar region.

Effects of the genes

(i) Mean vertebral width

The most striking effects were shown by *Sd* and *un*, with almost identical patterns in the two environments for each gene, and a degree of similarity of pattern between the two genes (Fig. 4). For *Sd* there was an overall tendency for the gene to produce a reduction in width at the cranial end of the series, gradually changing to an increase in width at the caudal end. Superimposed on this overall craniocaudal gradient were signs of resistance to the effect of the gene towards the vertebral class boundaries and in the mid-thoracic region. For *un*, the gene caused a dramatic general reduction in vertebral width (except for the second lumbar vertebra), with relative resistance to this effect at the cranial and caudal ends of the series and in the mid-thoracic region. There was some sign of resistance to size reduction at the T-L boundary, but little if any at the C-T boundary.

The effects of *T*, *Sp* and *Ph* are illustrated in Fig. 5. For each gene the starved mutant group tended to show greater vertebral width in relation to its own littermate control than the unstarved mutant group, indicating that these mutants enhanced the difference in mean

vertebral width observed between starved and unstarved wildtype controls. However, in other respects the three genes showed their own patterns of effects. The greatest difference in response between environments was shown by T where, in the starved environment, the pattern was suggestive of a craniocaudal gradient with relative stability towards most class boundaries. For Sp, in both environments, the gene resulted in a weak pattern of increasing size from the cranial and caudal extremes towards the centre of the complete series, with a mild suggestion of relative stability at the class boundaries. For Ph, in both environments, there was a weak overall tendency for the gene to reduce vertebral width progressively in a craniocaudal direction, again with a mild suggestion of relative stability at the class boundaries. More pronounced stability was observed in the mid-thoracic region.

There were no consistent effects of vt on mean vertebral width, with the mutant group values fluctuating between +1 and -1 per cent of those for their heterozygote controls in the two environments.

(ii) Variance of vertebral width

The most pronounced effects on the variance of vertebral width were shown by Sd, T and Ph (Fig. 6). For Sd, the gene in the normal environment produced a pattern of variances fluctuating widely around the control level. However, in the starved environment, Sd resulted in variances that were consistently and highly significantly increased over the control level, though with no vertebral class related pattern. For T, the gene in the normal environment tended to produce an increase in variance with a suggestion of a craniocaudal gradient, while in the starved environment variances were generally reduced below the control

values, fluctuating around the 95 and 99 per cent confidence limits in the thoracic segment. For Ph the reverse situation applied, with the gene in the normal environment reducing variance consistently below the 99 per cent confidence limit, while in the starved environment variances tended to be more like those of wildtype controls, although this effect was less pronounced in the C-T and T-L boundary regions. The variances of un homozygotes showed significant increases over their heterozygote control levels in the lumbar region for both environments, but there was no obvious effect elsewhere in the series. Variance patterns for Sp and vt showed no consistent or significant trends.

(iii) Instability variance

The instability variance ratio showed considerable fluctuations about unity, but nevertheless certain trends were apparent. For Sd in both environments the gene tended to cause a general increase of instability with more normal levels at the cranial and caudal extremes of the series. For Sp in both environments, the gene resulted in a craniocaudal gradient of increasing instability in the cervical class but not elsewhere in the series. The most clear-cut result was shown by un (Fig. 7) where, in both environments, the gene produced a surprising craniocaudal gradient of decreasing instability in the cervical class from a maximum level towards its cranial end to a more normal level at its caudal end, but a craniocaudal gradient of increasing instability in the thoracic class. There were no obvious patterns shown by the instability variance ratio for T, vt or Ph.

(iv) Directional asymmetry

No difference was noted between the pattern of directional asymmetry produced by the dominant genes and that shown by normal controls in either environment. However, vt and un caused marked disruption of the normal wildtype pattern illustrated in Fig. 3, both when homozygous and heterozygous. For each of these genes, patterns were similar for both genotypes and in both environments, but the homozygote pattern for the starved environment was perhaps the most pronounced. Homozygote patterns of directional asymmetry for the starved environment are therefore shown in Fig. 8, together with comparisons between these patterns and that shown by pooled normal wildtype controls from the dominant stocks. Both genes resulted in a widespread swing towards, or exaggeration of, right-sided predominance. For vt the normal cervical and thoracic peaks of left-sided predominance were lost and the normal lumbar right-sided predominance was enhanced. The difference between this pattern and that of normal wildtype controls showed resistance to the general effect of the gene at the C-T and T-L boundaries and for the first sacral vertebra. For un the normal cervical peak of left-sided predominance was less well defined and occurred more cranially, and increasing right-sided predominance was observed from the second thoracic vertebra to, and including, the lumbar segment. The difference between this pattern and that of normal wildtype controls showed that the effect of the gene was more pronounced towards the centre of the series, with relative resistance towards the cranial and caudal extremes.

The difference between these un and vt homozygote groups and normal wildtype controls in terms of mean vertebral width showed that un homozygote widths were generally less than those of controls (as they were compared with heterozygote widths, Fig. 4), while vt homozygote widths were generally greater than those of wildtype controls (even though there was no difference between homozygotes and heterozygotes). Since both homozygotes enhanced right-sided predominance, the smaller width for un must have been associated with preferential reduction of the left side, while the greater width for vt must have been associated with preferential enlargement of the right side.

DISCUSSION

A number of patterns were disclosed by normal wildtype controls in the unstarved environment, by starvation alone, and by the action of the different genes in the two environments. The five predominant patterns and their expression in the different groups are summarised in Table 4. In addition, there was the particular pattern of directional asymmetry that was common to all groups except vt and un heterozygotes and homozygotes.

In normal wildtype mice, the overall increase in the coefficient of variation of vertebral width with increasing distance from the cranial end of the series suggests a craniocaudal decrease in developmental control. A similar overall gradient might therefore be expected for the instability variance in normal mice, but this was not observed. However, it has been shown that different genetic systems may influence within individual variation (asymmetry) and between individual variation (Waddington, 1957, 1960; Reeve, 1960), and this may be responsible for differences of pattern shown by these two measures. On the other hand, both the coefficient of variation and the instability variance provided evidence of relative stability of the vertebral class boundary regions in normal mice (Fig. 2). The only exception was the high level of instability shown towards the caudal end of the lumbar region, which presumably was associated with the readiness of the background genotype to allow an increase in number of presacral vertebrae from 25 to 26, particularly as a result of starvation (Table 2). The right side was involved preferentially in this increase (Table 3), which is consistent with the right-sided predominance in directional asymmetry at the L-S boundary that was

found in almost all genotype/environment groups.

The response to 24-hour starvation on day 8 of gestation among wildtype controls showed an overall increase in vertebral width, previously reported to have followed starvation at earlier stages of pregnancy (Sofaer, 1978). This increase in size may have been the result of physiological overcompensation by the pregnant female following the resupply of food. The tendency for an increase in the instability variance, which might be expected, was not matched by an increase in the variance of vertebral width, which again may have an explanation in the different systems of control over within individual and between individual variation.

The response to starvation was generally rather uniform over the whole series; that is, there was no definite craniocaudal gradient and no clear vertebral class related pattern of response. The implication is therefore either that position in the series has no influence on the magnitude of the starvation effect, or that at the time of starvation the craniocaudal gradient and class boundaries have not yet become well established. However, two genes that are known to affect the very earliest stages of development of the axial skeleton (Sd and T, Table 1), stages that occur around day 8, produced patterns of effects showing both an overall craniocaudal gradient and relative stability of the class boundary regions (Table 4). Overall craniocaudal gradients of abnormality have previously been reported for both of these genes; for Sd in terms of vertebral size reduction, with maximum reduction at the cranial end of the series (cf Fig. 4), and for T in terms of increasing frequency of morphological anomalies (Grüneberg, 1963). Failure of starvation alone to disclose graded

and/or class related patterns of response is somewhat at variance with the mild indications of such responses reported previously (Sofaer, 1978). However, this difference may perhaps be partly attributable to background genotype, particularly since the total number of presacral vertebrae, which is known to be genotype dependent (e.g. Green, 1962), showed a much greater readiness to increase from 25 to 26 with starvation in the present material than in that reported previously.

The patterns observed for vt were a marked general increase in right-sided predominance, together with clear indications of resistance to this effect at the C-T and T-L boundaries (Fig. 8). This gene is known to affect the unsegmented paraxial mesoderm at around the same time that Sd and T have their effects on the truly axial structures. The disruption of normal developmental stability produced by these three genes therefore implies that both the axial and paraxial tissues acquire the potential for establishing vertebral class boundaries before segmentation occurs. The two patterns observed for Sp (Table 4), where segmentation may be affected secondarily to abnormal closure of the neural folds, both tend to confirm that the prospective class boundaries are established before segmentation.

The most marked and widespread effects of all were produced by un. This gene is known to influence sclerotome differentiation (Table 1), the process during which somites contribute to the early rudiments of the vertebrae. All patterns of disruption of normal developmental stability except an overall craniocaudal gradient were observed (Table 4).

The gene Ph, whose effects on the axial skeleton are indirect, through the development of hydrops around the notochord, resulted in

patterns of disruption of normal developmental stability that were similar to those shown by Sd and T, implying that both direct and indirect influences on the notochord can produce similar effects. A feature of the patterns shown by Ph, which was also present for Sd and un, was relative resistance to the effects of the genes in the mid-thoracic region. This is difficult to interpret in terms of vertebral class differentiation, but could conceivably be related to neighbouring cardiac development.

Reference to Table 4 shows that an overall craniocaudal gradient only occurred in response to genes affecting the primitive streak or notochord. Such a gradient is presumably some function of time and/or distance from the point of origin of the first axial structures, since the primitive streak starts to form at the cranial end and progresses towards the caudal end of the future axial skeleton, a process which takes approximately two days to reach the prospective lumbosacral region in the mouse. The gene vt, which affects the unsegmented paraxial mesoderm, and Sp and un, which have their effects after the onset of segmentation, did not produce a craniocaudal gradient of response, implying that the gradient is restricted to the truly axial structures and that after the onset of segmentation this information along the embryonic axis may be lost. This loss of overall craniocaudal gradient with segmentation is consistent with the passage of a wave of abrupt change in the presomite tissue that has been shown to confer resistance of segmental specification to the earlier disrupting effect of heat shock in amphibian embryos (Pearson & Elsdale, 1979). However, both Sp and un showed some signs of a residual craniocaudal gradient within vertebral classes.

Perhaps the most interesting finding is that the future vertebral class boundary regions appear to be established before visible signs of segmentation have occurred, since genes affecting the presegmentation stages (Sd, T, vt, Ph) produced vertebral class related patterns of disruption. This specificity of vertebral class boundaries prior to visible segmentation may be related to the metameric pattern of somitomeres in the presomitic mesoderm (Meier, 1979). Even at this stage, the somitomeres show signs of division into cranial and caudal halves, anticipating the contribution of each somite to the caudal and cranial halves of adjacent vertebrae which occurs during sclerotome differentiation (Solursh et al, 1979). Unlike the overall craniocaudal gradient, the special nature of the vertebral class boundary regions seems to persist, since genes affecting axial development after the onset of segmentation (Sp, un) also produced vertebral class related patterns of abnormality. The caudal and particularly the cranial extremes of the series appeared to be the regions of greatest stability in the column.

The evidence presented here for the establishment of vertebral class boundaries in the mouse before the onset of segmentation is supported by the results of direct experimental manipulation aimed at investigating regional determination in the vertebral column of chick embryos (Kieny et al, 1972). In these experiments, unsegmented presomitic mesoderm from the cervical region of a donor embryo was transferred to the thoracic region of a host embryo from which a corresponding piece of mesoderm had been removed. A similar transfer of unsegmented thoracic mesoderm was made to the cervical region. In both cases, the transplanted mesoderm developed according to its origin, resulting in either a rib-free area within the thoracic region or

supernumerary ribs in the cervical region of the column.

If the vertebral class boundaries are established before segmentation, the question of how they are established becomes analogous to the question of how somite size is regulated according to the size of the embryo. Somites are formed in a continuously growing system, the earliest appearing well before the embryonic axis is complete, so it is unlikely that their size is decided by a process of proportioning existing tissue (Flint et al, 1978). However, some mechanism is required whereby the correct number of somites of appropriate size is provided. There is persuasive evidence to suggest that the metameric pattern of somitomeres is generated at the very time when the paraxial mesoderm is being formed at the primitive streak, and that the amount of tissue incorporated into each prospective somite is determined by the rate of cell recruitment to the paraxial mesoderm and the rate of somite formation (Tam, 1981). At a different level of organisation, the C-T and T-L boundaries also appear to be specified before the embryonic axis is complete, so that the system must have some means of discriminating position in the series as the series is being formed. Specification of the L-S boundary has a ready explanation in the rather abrupt reversal of the somite size gradient observed in this region, presumably related to changes in the rates of cell recruitment and somite formation (Tam, 1981). However, the apparent specification of the C-T and T-L boundaries before axial development has reached the lumbosacral region remains, at least for the present, an enigma.

Acknowledgements

The work was supported by a project grant from the Medical Research Council. Miss Louise Anderson prepared the specimens and made the measurements.

Table 1. The six genes used, the site/nature of their abnormalities and the approximate gestational ages involved (Grüneberg, 1963). The differentiation of early axial structures takes place in a craniocaudal direction with about 2 days separating equivalent stages in the cranial and lumbosacral regions. AD = autosomal dominant, AR = autosomal recessive.

Gene	Symbol	Inheri-tance	Site/nature of abnormality	Approximate gestational age
Danforth's short tail	Sd	AD	Primitive streak or notochord	days 6½-8½
Brachyury	T	AD	Primitive streak or notochord	days 6½-8½
Vestigial tail	vt	AR	Unsegmented paraxial mesoderm	days 6½-8½
Splotch	Sp	AD	Segmentation (2ry to abnormal closure of neural folds)	days 8-10
Undulated	un	AR	Sclerotome differentiation	detectable effect from day 11
Patch	Ph	AD	Hydrops in association with notochord	most homozygotes die during days 9-10

Table 2. Numbers of mice with 25, 25½ or 26 presacral vertebrae in the different genotype/environment groups. The '25½' situation arises when there is asymmetrical articulation with the pelvis.

	Normal environment								Starved environment							
	Control				Mutant				Control				Mutant			
Gene	25	25½	26	% > 25	25	25½	26	% > 25	25	25½	26	% > 25	25	25½	26	% > 25
Sd	38	6	6	24	42	5	3	16	25	7	18	50	19	1	19	51
T	50	-	-	0	49	1	-	2	24	-	2	8	36	-	1	3
vt	49	-	1	2	47	3	-	6	37	9	4	26	39	3	3	13
Sp	50	-	-	0	49	1	-	2	31	2	17	38	22	6	9	41
un	2	1	47	96	8	6	36	84	1	-	49*	98	5	-	45	90
Ph	46	3	1	8	48	-	2	4	21	1	28	58	26	3	21	48

* Includes one mouse with 27 presacral vertebrae

Table 3. Numbers of mice of all genotype/environment groups for each mutant stock with 25½ presacral vertebrae showing the most caudal side of articulation with the pelvis ($\chi^2 = 13.5$, $p < 0.01$ for the overall difference between sides).

Stock	Left	Right
Sd	8	11
T	0	1
vt	4	11
Sp	2	7
un	1	6
Ph	0	7
Total	15	43

Table 4. A summary of the predominant patterns of instability observed for wildtype controls in the normal unstarved environment (N), due to starvation alone (S) and associated with the different genes in either or both environments. M or m, V or v, I or i, and D indicate that the pattern was observed in terms of Mean vertebral width, Variance of vertebral width (or coefficient of variation), the Instability variance, or Directional asymmetry respectively. Capital letters indicate a pronounced effect and lower case letters a mild effect.

Pattern	N	S	Sd	T	vt	Sp	un	Ph
Overall cranio-caudal gradient	V	-	M	m,v	-	-	-	m
Intraclass cranio-caudal gradient	M	-	-	-	-	i	I	-
Relative stability of class boundaries	V,I	-	M	m	D	m	m	m
Relative stability of mid-thoracic region	-	-	M	-	-	-	M	M,V
Whole series effects	-	M,V,i	V,i	v	D	-	M,D	V

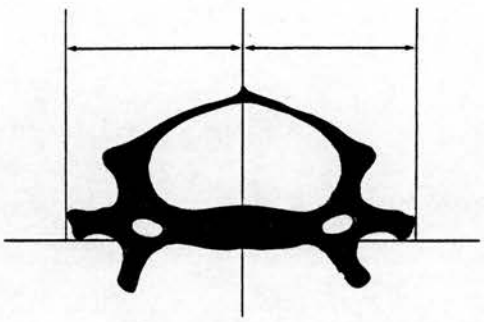


Fig. 1. Silhouette of a sixth cervical vertebra showing the two measurements made.

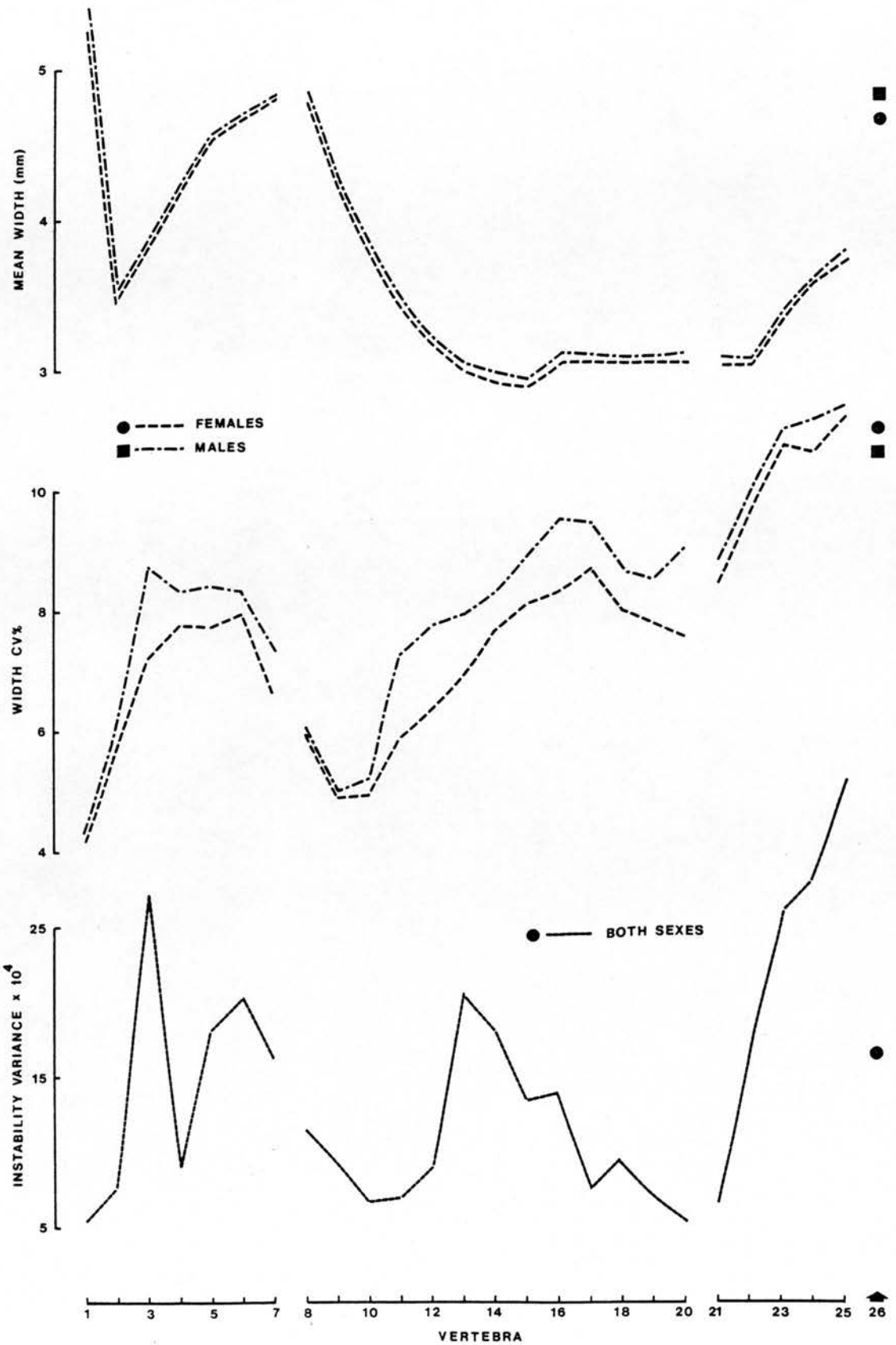


Fig. 2. Mean and coefficient of variation of vertebral width, and instability variance, for wildtype control mice in the normal unstarved environment (♀♀ = 97, ♂♂ = 103).

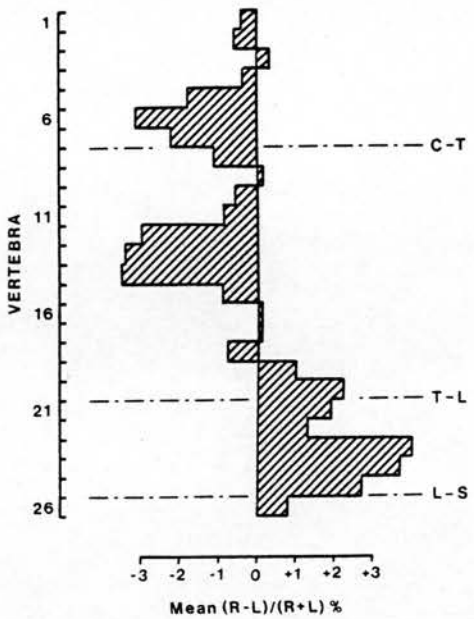


Fig. 3. Directional asymmetry, expressed as mean (R - L)/(R + L) per cent, for wildtype control mice in the normal unstarved environment (both sexes = 200). 21 of the 26 mean deviations differed significantly and 19/26 differed highly significantly from zero ($p < 0.05$ and $p < 0.01$ respectively).

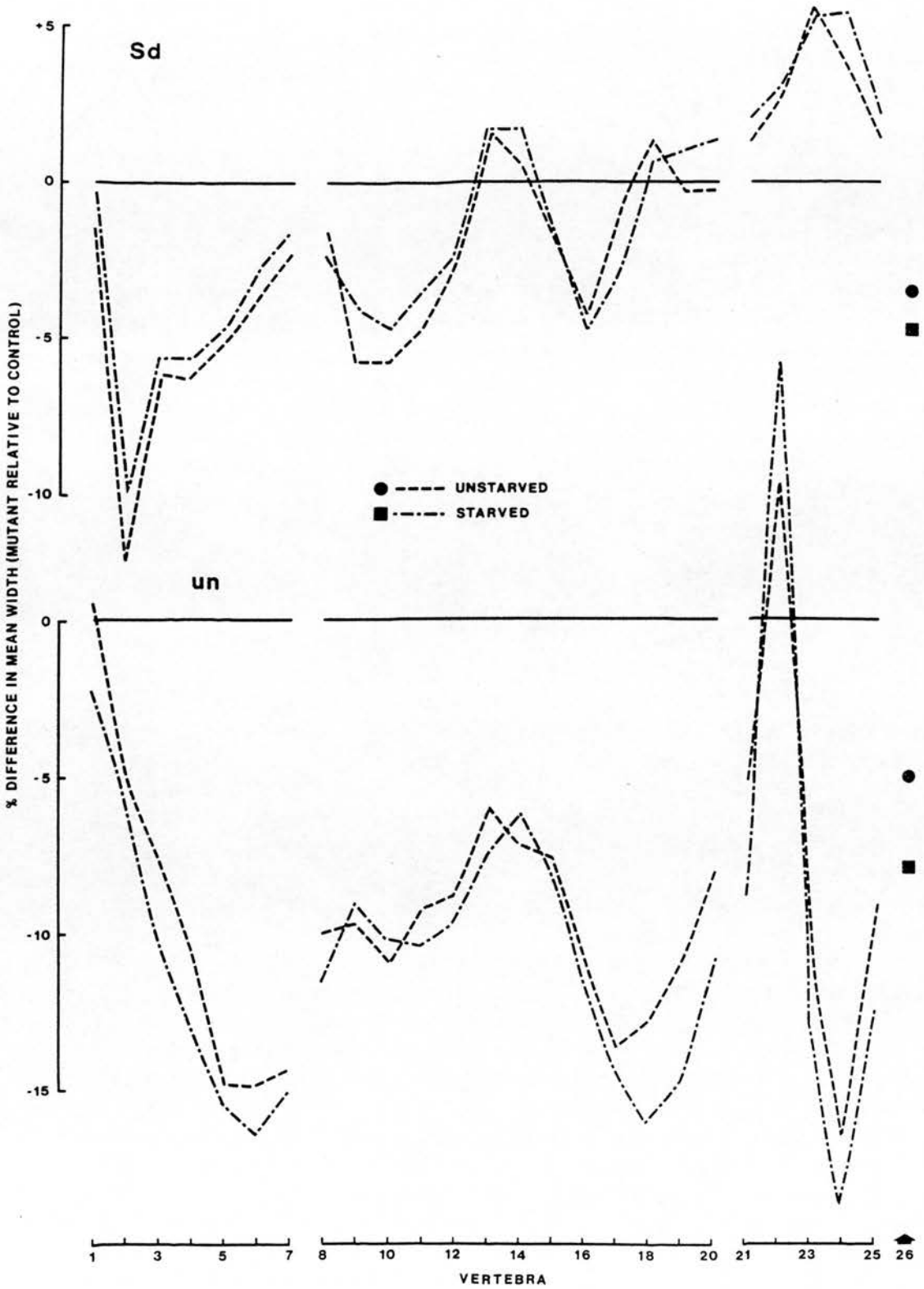


Fig. 4. Effects of the genes Sd and un on mean vertebral width in normal and starved environments, expressed as the percentage difference in width shown by the mutant groups relative to their littermate controls.

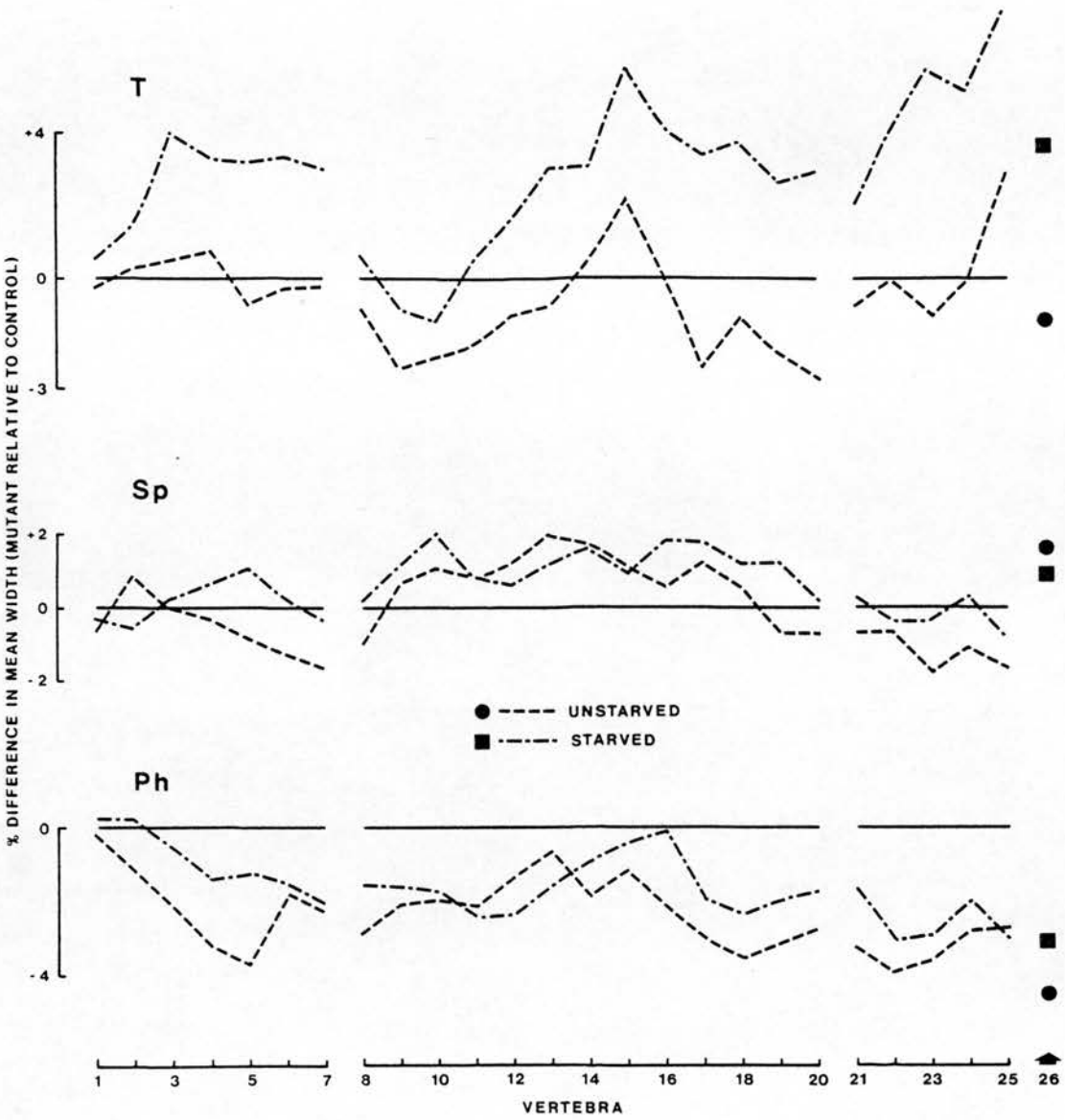


Fig. 5. Effects of the genes T, Sp and Ph on mean vertebral width in the normal and starved environments, expressed as the percentage difference in width shown by the mutant groups relative to their littermate controls.

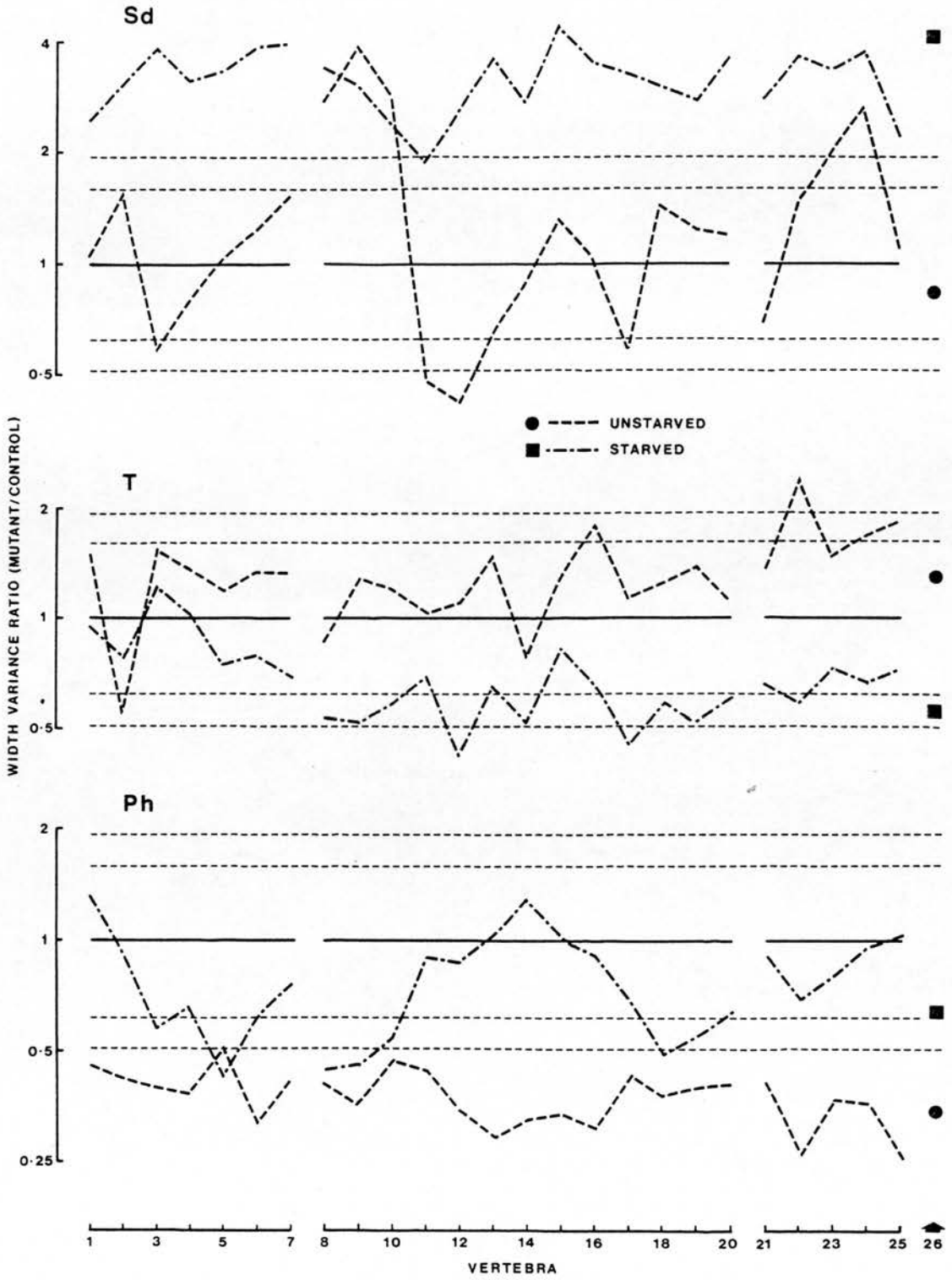


Fig. 6. Effects of the genes Sd, T and Ph on the variance of vertebral width, expressed as the ratio of mutant variance to littermate control variance in the two environments. The horizontal broken lines indicate the 95 and 99 per cent confidence limits for individual variance ratios.

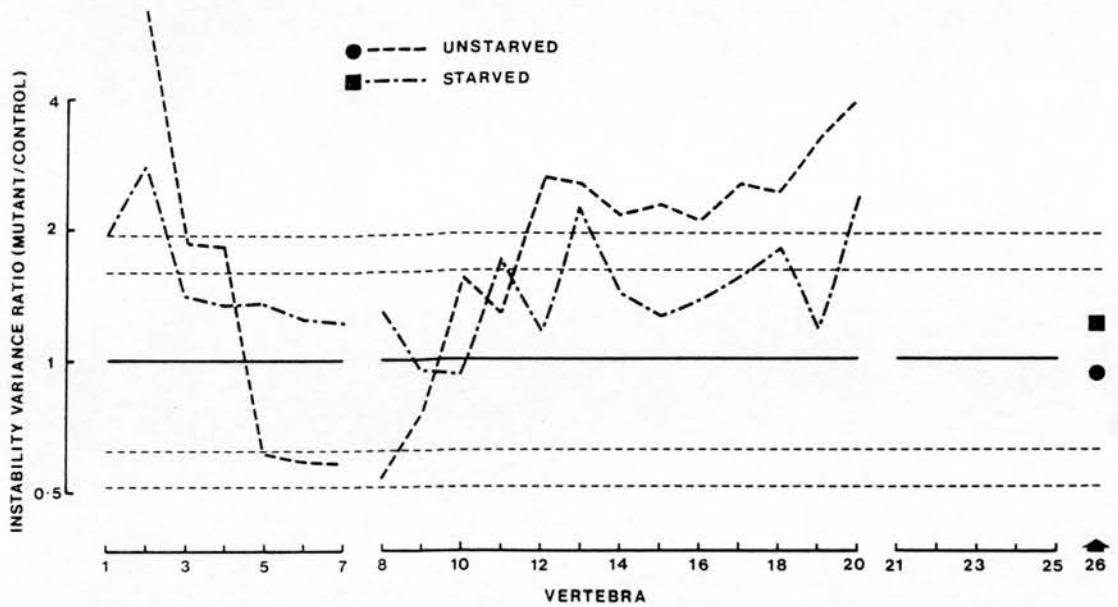


Fig. 7. Effects of the gene un on the instability variance, expressed as the ratio of mutant variance to control variance in the two environments. The horizontal broken lines indicate the 95 and 99 per cent confidence limits for individual variance ratios. The plots are incomplete because of some negative estimates for the instability variance in one or other of the genotype/environment groups.

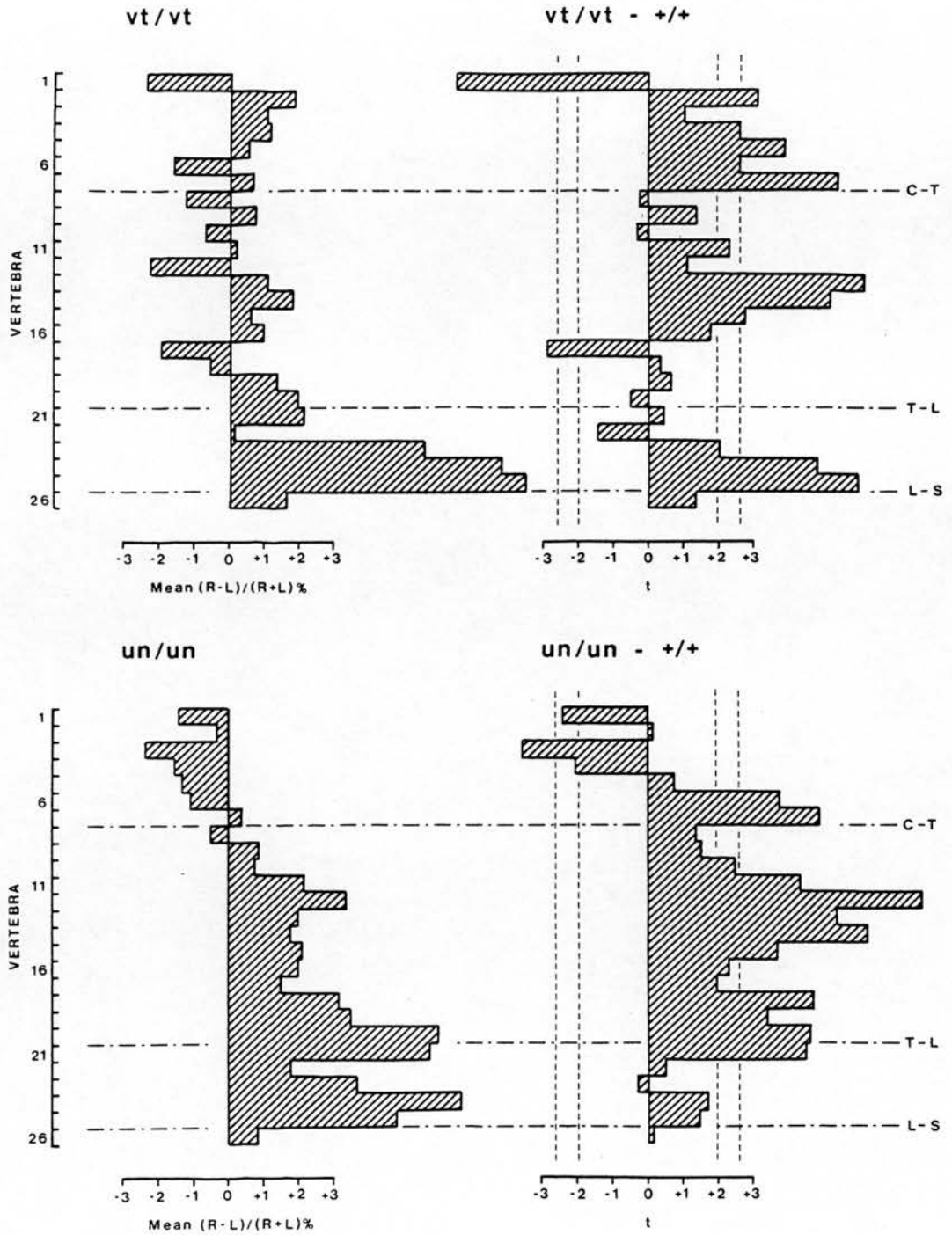


Fig. 8. Effects of the genes vt and un on directional asymmetry, expressed both as mean (R - L)/R + L) per cent for homozygotes in the starved environment, and in terms of t values showing the direction and statistical significance of the difference between these mean values and those of wildtype controls for the dominant stocks in the normal environment (Fig. 3). The broken lines indicate the 95 and 99 per cent confidence limits for individual mean comparisons.

REFERENCES

Butler, P.M. (1967). Dental merism and tooth development. J. dent. Res. 46, 845-850.

Dagg, C.P. (1966). Teratogenesis. In Biology of the Laboratory Mouse (ed. E.L. Green), pp. 309-328. New York, Toronto, Sydney, London: McGraw-Hill.

Flint, O.P., Ede, D.A., Wilby, O.K. & Proctor, J. (1978). Control of somite number in normal and amputated mouse embryos: an experimental and theoretical analysis. J. Embryol. exp. Morph. 45, 189-202.

Green, E.L. (1962). Quantitative genetics of skeletal variations in the mouse. II. Crosses between four inbred strains. Genetics 47, 1085-1096.

Grüneberg, H. (1963). The Pathology of Development. Oxford: Blackwell.

Kieny, M., Mauger, A. & Sengel, P. (1972). Early regionalisation of the somitic mesoderm as studied by the development of the axial skeleton of the chick embryo. Devl Biol. 28, 142-161.

Lumsden, A.G.S. (1979). Pattern formation in the molar dentition of the mouse. J. Biol. buccale 7, 77-103.

Luther, P.G. (1949). Enzymatic maceration of skeletons. Proc. Linn. Soc. 161, 146-147.

Meier, S. (1979). Development of the chick mesoblast: formation of the embryonic axis and establishment of the metameric pattern. Devl Biol. 73, 25-45.

Osborn, J.W. (1978). Morphogenetic gradients: fields versus clones. In Development, Function and Evolution of Teeth (ed. P.M. Butler & K.A. Joysey), pp. 171-201. London: Academic Press.

Pearson, M. & Elsdale, T. (1979). Somitogenesis in amphibian embryos. I. Experimental evidence for an interaction between two temporal factors in the specification of somite pattern. J. Embryol. exp. Morph. 51, 27-50.

Reeve, E.C.R. (1960). Some genetic tests on asymmetry of sternopleural chaeta number in Drosophila. Genet. Res., Camb. 1, 151-172.

Sofaer, J.A. (1978). Morphogenetic influences and patterns of developmental stability in the mouse vertebral column. In Development, Function and Evolution of Teeth (ed. P.M. Butler & K.A. Joysey), pp. 215-227. London: Academic Press.

Solursh, M., Fisher, M., Meier, S. & Singley, K.T. (1979). The role of extracellular matrix in the formation of the sclerotome. J. Embryol. exp. Morph. 54, 75-98.

Tam, P.P.L. (1981). The control of somitogenesis in mouse embryos. J. Embryol. exp. Morph. 65 (Supplement), pp. 103-128.

Van Valen, L. (1970). An analysis of developmental fields. Devl Biol. 23, 456-477.

Waddington, C.H. (1957). The Strategy of the Genes. London: George Allen and Unwin.

Waddington, C.H. (1960). Experiments on canalising selection. Genet. Res., Camb. 1, 140-150.

DENTAL DEVELOPMENT AND EVOLUTIONARY CHANGE

Reduction in size of the jaws during hominid evolution has been accompanied by a general reduction of tooth size, but within each morphological class the later a tooth develops the more it has been reduced. Within each class, greater evolutionary reduction is also associated with greater phenotypic variability in contemporary populations, and this greater variability appears to be due to a larger environmental component. The apparent paradox of this association between more rapid evolutionary change and greater non-genetic variability can be explained on a developmental basis involving compensatory interaction between early and late developing teeth of the same class under the constant restricting pressure of a changing skeletal system.

A DEVELOPMENTAL BASIS FOR DIFFERENTIAL TOOTH REDUCTION DURING HOMINID EVOLUTION

J. A. Sofaer,[1] H. L. Bailit,[2] and C. J. MacLean[1]

[1]*Human Genetics Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014, and* [2] *University of Connecticut, Health Center, Farmington, Connecticut 06032*

Received December 1, 1970

Reduction in size of the jaws during hominid evolution has been accompanied by a general reduction of tooth size. Natural selection has presumably operated to maintain a harmonious tooth to jaw size relationship by tending to eliminate genotypes that produced teeth too large for the changing skeletal system. An interesting feature of the reduction process is the more rapid change that has occurred in the most posterior teeth of each jaw. Taking *Australopithecus* as representative of the early hominid condition, the data presented by Robinson (1954), for the tooth size module *(length + width)/2* in the lower jaw, show a clear pattern of reduction in the change from early hominid to modern man. Not only has size reduction been greater in the most posterior teeth of the jaw as a whole, but the most posterior members of each morphological class (incisors, premolars and molars) appear to have been reduced more rapidly than their anterior neighbors. The pattern of reduction found by Robinson in the upper jaw was apparently very similar. The data presented by Brace (1967), using the module *length* × *width* for the upper teeth, show the same pattern for the premolars and molars.

Robinson (1954) postulated that the more rapid reduction of the second and third molars has been secondary to restriction of space due to skeletal reduction. The third molar was presumed to be the most vulnerable because it is the last tooth to erupt and must therefore fit into the space that remains after all the other teeth in the jaw have come into functional occlusion. However, the most posterior position and time of eruption of the second and third molars are not alone sufficient reasons for the more rapid change that has occurred in these teeth. For example, malposition of the third molar due to insufficient space would not result simply in selection against large third molars but against large teeth in general, since lack of space for the third molar depends on the sizes of all the teeth in the jaw. Furthermore, there is some evidence from the mouse and man to suggest that genetic variation forms the lowest proportion of total size variation in the most posterior tooth of each class (Bader and Lehmann, 1965; Bader, 1965; Lundström, 1948; Hunter, 1959); a condition which, all other things being equal, would result in a less rapid response to selection in these teeth.

Nevertheless, the time at which each tooth develops does seem to be related to the amount of reduction it has undergone. Figure 1 shows the reduction that has taken place from *Australopithecus* to modern man, the data being taken from Robinson (1954); and the time of the first appearance of hard tissue in each developing tooth (Orban, 1957). The correspondence between the evolutionary reduction and developmental timing patterns is clear. Except in the case of the lower incisors, which develop at very much the same time, the later the development of a tooth within a morphological class the more it has been reduced.

The purpose of this paper is to examine aspects of variation in the dentition of a sample of modern man, with particular reference to the way in which tooth size can be modified by the local environment

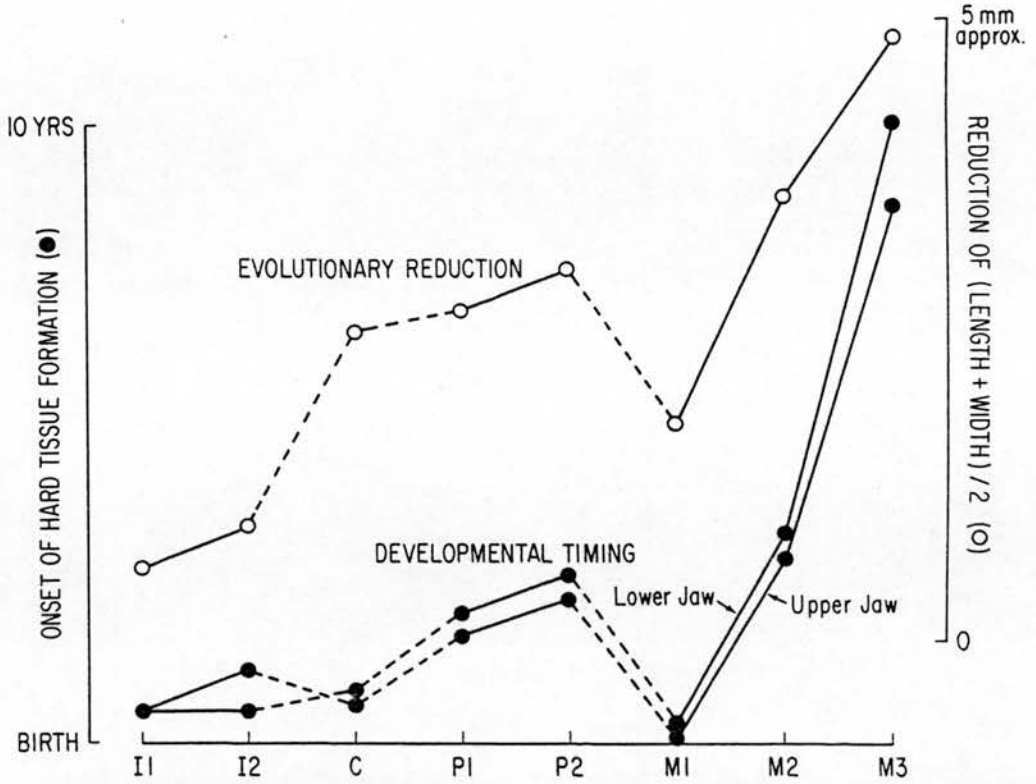


FIG. 1. The pattern of evolutionary reduction from *Australopithecus* to modern man, and the pattern of developmental timing (Orban, 1957), for incisors (I1 and I2), canine (C), premolars (P1 and P2), and molars (M1, M2 and M3). The solid lines connect points for teeth of the same class, and the broken lines connect points for adjacent teeth of different classes. The pattern of evolutionary reduction was obtained from Robinson's (1954) Figure 1 by subtraction of the module values plotted for modern man ("White") from those plotted for *Australopithecus*.

during development. The analysis is largely concerned with making comparisons between early and late developing teeth of the same class. An explanation is then advanced, in terms of space restriction and developmental timing, for the differential size reduction that has taken place during hominid dental evolution.

MATERIALS AND METHODS

The data were derived from an examination of dental casts of two Melanesian tribes: Nasioi, collected on the island of Bougainville in the Territory of Papua and New Guinea; and Kwoio, collected on Malaita, one of the British Solomon Islands. These two tribes have been the subject of other recent investigations (Bailit et al., 1968; Friedlander and Bailit, 1969; Bailit et al., 1970). Each individual had at least one first degree relative in the sample, the total of 229 individuals yielding 117 parent-offspring pairs and 146 sibling pairs for both tribes combined. Mesiodistal and buccolingual tooth diameters were measured to the nearest .05 mm using a Helios Dial Caliper, model VD6HC, with specially pointed beaks. All teeth except the canines are considered here.

Three aspects of size variation were investigated. Firstly, coefficients of variation for each tooth and phenotypic correlations between all pairs of teeth within each jaw were calculated. The results of these calculations simply gave a general picture of the pattern of variation in the dentition

TABLE 1. *Mean tooth size in millimeters and its standard error, and the coefficient of variation (C.V., %), for mesiodistal and buccolingual diameters of all teeth except the canines. N is the number of individuals, each of whom was scored either as the average of measurements on the right and left sides, or, if only one side was measured, as the measurement on that side.*

	Mesiodistal Diameter			Buccolingual Diameter		
	N	Mean ± S.E.	C.V.	*N*	Mean ± S.E.	C.V.
UI1	216	8.83 ± .04	7.5	167	7.74 ± .05	9.1
UI2	217	7.51 ± .05	9.8	193	6.89 ± .05	9.5
UP1	214	7.46 ± .04	7.3	210	10.28 ± .05	6.7
UP2	207	7.00 ± .04	7.3	204	10.25 ± .05	7.2
UM1	222	10.80 ± .04	5.5	220	12.05 ± .05	6.1
UM2	184	10.13 ± .06	7.5	191	12.17 ± .06	7.0
UM3	103	8.93 ± .09	10.2	106	11.35 ± .12	11.3
LI1	209	5.63 ± .03	7.0	138	6.54 ± .06	11.0
LI2	213	6.33 ± .03	7.0	168	6.75 ± .05	9.8
LP1	210	7.36 ± .04	7.7	198	8.57 ± .04	7.3
LP2	207	7.43 ± .05	9.1	195	9.07 ± .05	7.2
LM1	221	11.54 ± .04	5.7	213	11.13 ± .04	5.6
LM2	194	10.84 ± .06	7.1	186	10.98 ± .05	6.7
LM3	100	10.81 ± .09	8.6	94	10.52 ± .10	8.8

TABLE 2. *Correlations of (R+L)/2 between pairs of teeth within each jaw for the mesiodistal diameter (upper right) and the buccolingual diameter (lower left half of each matrix).*

	UI1	UI2	UP1	UP2	UM1	UM2	UM3
UI1	—	.67	.55	.49	.59	.38	.31
UI2	.75	—	.54	.50	.44	.37	.17
UP1	.49	.44	—	.78	.53	.53	.48
UP2	.44	.31	.84	—	.54	.65	.47
UM1	.59	.47	.72	.70	—	.51	.47
UM2	.56	.55	.70	.73	.79	—	.50
UM3	.56	.20	.64	.62	.63	.71	—
	LI1	LI2	LP1	LP2	LM1	LM2	LM3
LI1	—	.74	.44	.37	.55	.41	.23
LI2	.87	—	.60	.45	.59	.47	.33
LP1	.51	.60	—	.62	.58	.61	.54
LP2	.48	.55	.84	—	.42	.64	.44
LM1	.35	.40	.67	.70	—	.68	.48
LM2	.43	.46	.61	.64	.81	—	.49
LM3	.40	.24	.51	.48	.49	.81	—

as a whole and of the relationships of one tooth to another. Secondly, the analysis was concerned with asymmetry, as measured by (R–L)/(R+L), where R and L are one individual's tooth measurements on the right and left sides. The variable (R–L)/(R+L) is an expression of asymmetry corrected for tooth size, allowing meaningful comparisons to be made between large and small teeth. The variance of (R–L)/(R+L) was calculated for each tooth, and the more anterior and more posterior teeth of each class were compared using a variance ratio test. Correlations between all teeth within each jaw were also calculated for (R–L)/(R+L). Since the genetic size potentials of the two sides of the same individual can be regarded as identical, the asymmetry results gave some indication of how different teeth may be influenced by local environmental effects during development. Finally, the degree of resemblance between relatives was estimated by the intra-class correlation for each measurement among all parent-offspring pairs and all sibling pairs. Comparisons of different teeth with respect to the degree of resemblance between relatives were then carried out to detect differences in the extent of genetic control over tooth size between teeth. For this analysis all female measurements were increased by 4%, since a regression of tooth size on sex showed male measurements to be approximately 4% larger on average.

RESULTS

Table 1 shows the mean mesiodistal and buccolingual diameters for the different teeth, and the coefficients of variation for each measurement in each tooth. There is no question about the relative variability of the molars. For both measurements in both upper and lower jaws the later the development of the molar the greater its variability. From Figure 1 it can be seen that the greatest difference of developmental timing between teeth of the same class is shown by the molars. The next greatest difference is shown by the premolars and upper incisors, and the lower incisors show no difference of any significance. The relationship between variability and developmental timing within a tooth class is therefore reasonably con-

TABLE 3. *Comparison of the variance of (R–L)/(R+L) between members of the same tooth class, for mesiodistal and buccolingual diameters.*

	Upper Jaw				Lower Jaw			
	Mesiodistal		Buccolingual		Mesiodistal		Buccolingual	
	Var. ($\times 10^4$)	F	Var. ($\times 10^4$)	F	Var. ($\times 10^4$)	F	Var. ($\times 10^4$)	F
I1	1.43		2.46		3.46		4.02	
		2.39**		27.09**		0.88		1.01
I2	3.42		66.65		3.05		4.08	
P1	2.72		1.81		4.26		2.88	
		1.07		1.97**		0.78†		1.11
P2	2.91		3.56		3.34		3.19	
M1	1.51		3.00		1.13		2.10	
		3.35**		1.19		1.70**		1.92**
M2	5.06		3.58		1.92		4.04	
M1	1.51		3.00		1.13		2.10	
		6.03**		29.60**		4.25**		53.40**
M3	9.10		88.81		4.80		112.13	
M2	5.06		3.58		1.92		4.04	
		1.80**		24.81**		2.50**		27.75**
M3	9.10		88.81		4.80		112.13	

* $P \leq .05$, ** $P \leq .01$.
† $P \leq .05$ with the more anterior tooth more asymmetrical.

sistent over all classes, since the differences between early and late developing premolars and upper incisors are not as marked as between the molars, and since the lower incisors show no difference between central and lateral with respect to the mesiodistal diameter. On the other hand, the lower central did show greater variability than the lateral with respect to the buccolingual diameter. It should be mentioned here, however, that the buccolingual diameter of the lower incisors is perhaps not a very reliable measurement since the accumulation of salivary calculus, which may not be noticed on a cast, is likely to be a source of error.

Table 2 presents correlations for the mean measurement of right and left sides for each tooth between pairs of teeth within each jaw. The highest coefficients are between members of the same tooth class. Between tooth classes the highest coefficients are between the earliest developing members. These findings are in agreement with those of Garn, Lewis and Kerewsky (1965), and suggest a decrease of intrinsic control over tooth size from early to late within each class.

Table 3 lists comparisons of the variance of (R–L)/(R+L) between members of the same tooth class. Except for the lower premolars there is little doubt that within each class the later a tooth develops the more asymmetry it expresses. This holds for both dimensions, though the difference between early and late appears on average to be greater for the buccolingual diameter. The lower incisors show no differences, but again they show little or no difference of developmental timing.

Table 4 presents correlations of (R–L)/(R+L) between pairs of teeth within each jaw. The difference between the two dimensions is striking. There are many significant positive coefficients for the buccolingual diameter but few for the mesiodistal diameter. Further, the mesiodistal diameter shows a large number of negative coefficients. Positive correlation of (R–L)/(R+L) between two teeth indicates that they tended to be larger on the same side of the jaw. Positive correlation can there-

TABLE 4. *Correlations of (R-L)/(R+L) between pairs of teeth within each jaw for the mesiodistal diameter (upper right) and the buccolingual diameter (lower left half of each matrix).*

	UI1	UI2	UP1	UP2	UM1	UM2	UM3
UI1	—	.04	.22**	.03	.07	.15	.06
UI2	.08	—	.02	−.14	.05	0	−.03
UP1	.12	.21*	—	.08	.11	−.01	.14
UP2	−.02	.37**	.51**	—	−.16*	.06	.31*
UM1	−.04	.03	.30**	.46**	—	−.05	−.25
UM2	−.14	.25**	.20*	.43**	.52**	—	.22
UM3	.01	.26	.33**	.17	.19	.28*	—
	LI1	LI2	LP1	LP2	LM1	LM2	LM3
LI1	—	.23**	−.10	.08	−.02	.09	.29*
LI2	.01	—	.10	.02	−.07	.19*	.24
LP1	−.08	.24*	—	0	.02	.04	−.18
LP2	.11	.11	.27**	—	−.06	.05	.03
LM1	.08	.07	.18*	.19*	—	−.08	.05
LM2	.20	.09	.11	.23**	.55**	—	.30*
LM3	.17	.45*	.37**	.15	−.11	.28*	—

* $P \leq .05$, ** $P \leq .01$

fore be interpreted as being due to responses to common local environmental conditions affecting neighboring teeth on the same side of the jaw. On the other hand, negative correlation implies the existence of compensatory interaction between adjacent teeth. That is, in a segment of say two teeth, a larger than average first tooth would tend to be associated with a smaller than average second tooth, and vice versa. Since any local environmental conditions causing the size of neighboring teeth to be positively correlated presumably have the potential to affect both tooth dimensions equally, the difference between the buccolingual and mesiodistal diameters can be taken as representing a dampening of the reaction to the common local environment by compensatory interaction along the length of the jaw.

Correlations between pairs of relatives are shown in Table 5. The *t* values indicate the significance and direction of the differences between correlations for siblings and correlations for parents and their offspring. The pattern of values again illustrates that the two dimensions do not react equally to all conditions. The mesiodistal diameter shows no meaningful differences between the two kinds of correlations, though the majority of *t* values are negative. By contrast, for the buccolingual

TABLE 5. *Correlations between relatives.*

	Mesiodistal Diameter					Buccolingual Diameter				
	Sibs		Parents & Offspring			Sibs		Parents & Offspring		
Tooth	*N*	*r*	*N*	*r*	*t*	*N*	*r*	*N*	*r*	*t*
UI1	125	.51	99	.42	.88	84	.09	48	.11	−.11
UI2	127	.39	102	.44	−.45	108	.31	71	.17	.90
UP1	124	.45	92	.59	−1.46	120	.44	88	.39	.45
UP2	111	.40	83	.49	−.80	112	.30	80	.27	.27
UM1	136	.49	107	.54	−.46	137	.43	105	.31	1.01
UM2	96	.31	56	.29	.14	106	.42	60	.26	1.09
UM3	45	.28	18	.47	−.77	51	.21	19	.25	−.16
LI1	126	.27	88	.15	.92	64	.33	37	−.06	1.89
LI2	129	.28	93	.33	−.44	83	.38	48	.09	1.67
LP1	121	.55	88	.51	.34	103	.33	78	.26	.54
LP2	114	.18	79	.35	−1.25	105	.41	68	.12	1.99*
LM1	131	.27	102	.24	.22	126	.41	94	.22	1.58
LM2	100	.22	67	.43	−1.45	97	.44	64	.27	1.15
LM3	47	.09	15	.50	−1.43	46	.12	16	.60	−1.83

N is the number of pairs of relatives that contributed to the calculation of *r*, the correlation coefficient. The value and sign of *t* indicate the significance and direction of the differences of correlations for parents and their offspring from those of siblings.

* $P \approx .05$, $d.f. = \infty$.

TABLE 6. *Correlations between all pairs of first degree relatives showing the differences between the more anterior and more posterior teeth within each class.*

Tooth Pair	Mesiodistal Diameter			Buccolingual Diameter		
	Ant.	Post.	t	Ant.	Post.	t
UI1 and UI2	.47	.42	.75	.10	.26	−1.40
UP1 and UP2	.51	.44	.95	.42	.29	1.48
UM1 and UM2	.51	.30	2.43*	.38	.37	.16
UM1 and UM3	.51	.33	1.54	.38	.22	1.28
UM2 and UM3	.30	.33	−.20	.37	.22	1.11
LI1 and LI2	.22	.30	−.88	.20	.29	−.68
LP1 and LP2	.53	.25	3.32**	.30	.31	−.09
LM1 and LM2	.26	.31	−.55	.33	.38	−.50
LM1 and LM3	.26	.18	.52	.33	.25	.61
LM2 and LM3	.31	.18	.88	.38	.25	.93

The value and sign of t indicate the significance and direction of the difference of posterior from anterior.

* $P \leq .05$, ** $P \leq .01$, $d.f. = \infty$.

diameter, the differences are on average definitely positive, suggesting possible effects of dominance and common sibling environment (Falconer, 1964). However, since any such effect must have been small, the correlations between siblings and those between parents and their offspring were pooled to give a single set of correlations for first degree relatives.

Correlations between first degree relatives, comparing the more anterior and more posterior teeth within each class, are shown in Table 6. The t values indicate the significance and direction of the difference between anterior and posterior. Only two t values are significant, but these are both positive. The remaining t values, though not high, are positive on average. Disregarding the lower incisors, the general suggestion is then that relatives tend to be more alike with respect to the more anterior, earlier developing, tooth in each class than with respect to the more posterior, later developing tooth. The proportion of the total size variation due to genetic causes would therefore seem to be lowest in the latest developing tooth of each class. The difference between anterior and posterior does however appear to be more convincing for the mesiodistal than for the buccolingual diameter. This could perhaps be due to the more pronounced reaction to common local environment shown by the buccolingual diameter (Table 4).

Though none of these results alone can be regarded as conclusive, the overall picture, interpreted in the light of previous work, indicates that the greater variability observed in the later developing tooth of each class is due to greater environmental variation, and that this environmental variation is partly the result of local effects related to compensatory interaction between teeth developing in a confined space.

DISCUSSION

In addition to the antero-posterior developmental sequence within each tooth class, Figure 1 shows that the most posterior member of each class starts to develop after the most anterior member of the class distal to it (except in the case of the lower incisors). The most posterior tooth of each class is therefore usually the last to start developing in its region of the jaw, and must therefore make do with what remains of any local requirements that are necessary for development. Thus it is likely, simply on developmental grounds, that the most posterior tooth of each class will reflect most the effects of variation in the amount of available space.

It has in fact long been recognized that the last tooth to develop in each class tends to be the most variable (Dahlberg, 1945). The relationship between developmental timing and coefficients of variation in Table 1 is intended as a confirmation of this fact in the present sample. That this greater variability is due to a higher environmental component is suggested by the apparent falling off of intrinsic control over tooth size from the early to the late developing teeth within each class (Table 2); and by the greater asymmetry (Table 3), and the tendency towards lower correlation between relatives (Table 6) shown by the later developing teeth.

The expression of environmental variation seen in the buccolingual diameter appears however to be modified in the mesiodistal diameter, presumably by local interactions between teeth developing in a space that is restricted mesiodistally. Differences between the two dimensions are shown with respect to asymmetry differences between the more anterior and more posterior teeth of each class (Table 3), asymmetry correlations between teeth (Table 4), correlations between different kinds of relatives (Table 5), and differences with respect to correlations between relatives between the more anterior and more posterior teeth of each class (Table 6). This evidence for local interactions between teeth in a confined space is in fact supported by other studies that have pointed towards the existence of compensatory interaction between tooth germs during development. The findings of these studies indicate that if in a given morphological class the teeth which develop early are large, then those which develop late tend to be small or absent, and vice versa (Grüneberg, 1951; Grewal, 1962; Van Valen, 1962; Sofaer, 1969; Sofaer et al., 1971).

Selection acts on the phenotype, but the response to selection depends on the extent to which the phenotype reflects the genotype. The role of environmental factors in modifying the development of the phenotype is therefore an important consideration when evaluating the basis of any evolutionary change. The results presented here, taken in conjunction with the results of previous work, indicate that different teeth are subject to different local environmental conditions, and the extent to which the final phenotype reflects the genotype differs from one tooth to another. It is therefore easy to understand how the response to selection for tooth size, under the pressure of the changing skeletal system, could differ among teeth. However, the teeth that have changed most rapidly appear to have phenotypes that are the poorest reflection of the genotype, or at least no better than any other.

Assuming that dental change has been largely secondary to skeletal change, the following conclusions can be drawn. Since the dental changes must have lagged behind the skeletal changes the teeth must have always been, on average, genetically too large for the jaw in which they developed. Environmental deviations, expressed most by the latest tooth to develop in each class, would then have been overwhelmingly negative due to the restriction of space. Lack of harmony between tooth size and jaw size would therefore have been the result of inability of the individual to respond sufficiently by negative environmental deviations, and selection would have operated against genotypes that failed to produce negative deviations large enough to accommodate all the teeth comfortably in the restricted jaw.

If there was no difference between jaw size and tooth size potential then negative deviations would not be necessary to accommodate the teeth. It is therefore reasonable to suppose that the size of the negative deviation expressed by any late developing tooth would somehow be related both to the genetic size potential of the tooth and to the amount of space available. One possible relationship would depend on the ratio of the size potential of the tooth to the available space. On this

TABLE 7. *The four possible combinations of "genotypically small" and "genotypically large" teeth (arbitrarily defined by values of 3 and 4 respectively) in a hypothetical class of two. The first tooth to develop is assumed to be unaffected by any directional environmental bias, whereas the second tooth to develop shows a negative environmental deviation, the size of which is equal to some constant proportion,* x *(say* $x = \frac{2}{3}$*), of the ratio of the size potential of the second tooth,* G, *to the space available,* S. *The total space allotted to the two teeth is assumed to be 5 units.*

1st Tooth Genotype & Phenotype	2nd Tooth Genotype (= G)	Space remaining after development of 1st Tooth (= S)	Phenotype of 2nd Tooth (= G − x(G/S))	Combined Phenotype (1st + 2nd)
3	3	2	2	5
3	4	2	2.67	5.67
4	3	1	1	5
4	4	1	1.33	5.33

basis, a tooth whose size potential was, say, three times as large as the available space would suffer greater reduction than a tooth whose size potential was only twice as large as the available space.

The above relationships are illustrated in Table 7. Listed are the four possible combinations of "genotypically small" and "genotypically large" teeth (arbitrarily defined by values of 3 and 4 respectively) in a hypothetical class of two. The first tooth to develop is assumed to be unaffected by any directional environmental bias, so the phenotype is, on average, the same as the genotype. The second tooth to develop shows a negative environmental deviation, the size of which is equal to $x(G/S)$, where x is a constant, G is the genetic size potential of the second tooth, and S is the space available.

The two combinations of genotypes in Table 7 that are critical to the present argument are those in which one of the two teeth is genotypically large and the other genotypically small. Selection for or against a large-small combination would tend to change the relative frequency of large genotypes in the two teeth and thus produce a differential change of mean size potential. Selection for or against the large-large combination or the small-small combination would not result in such a size differential. The proposed relationship between the phenotype of the second tooth and its genotype and the space available shows how the two critical combinations, in which the combined genetic potentials of the two teeth are identical, can produce different combined phenotypes. The smallest combined phenotype of the two, which because of the reducing skeletal system can be regarded as selectively the most advantageous, results from a genotypically large first tooth and a genotypically small second tooth. Thus, on the basis of the model illustrated in Table 7, there could be a tendency for the first tooth to remain large but for the second tooth to become reduced through selection for tooth to jaw size harmony.

Table 7 obviously illustrates a specific and simplified situation. However, on the assumption that the negative deviation in the second tooth is proportional to some function of G and S, there would only be one case (in which the phenotype of the second tooth $= G - x(G - S)$) where a phenotype differential between the two critical combinations would not occur. Very many different functions of G and S would therefore produce a phenotype differential in the postulated direction, and even a relatively small differential could conceivably be sufficient to result in a difference of selective advantage between the two critical combinations. The apparent paradox of the association of more rapid evolutionary change with greater non-genetic variation can therefore be explained in terms of developmental sequence and the response to restriction of space.

It is not intended to imply that the mechanism proposed here has been responsible for all the changes that have taken place during hominid dental evolution. Reference to Figure 1 shows that even though the within tooth class changes could be reasonably explained largely on this basis, the between tooth class changes ap-

pear to have involved another factor. The first molar, which is one of the first permanent teeth to develop in the whole jaw, has undergone considerably more reduction than the incisors which develop at around the same time. This additional factor has presumably been related more directly to the teeth through the changing use to which they themselves have been subjected. However, it does seem that effects purely secondary to skeletal reduction could have provided a relatively potent force in producing differential tooth reduction during hominid evolution.

Summary

Reduction of tooth size during hominid evolution has been greater in the most posterior teeth of each jaw as a whole, and within each tooth class (incisors, premolars and molars) the later developing teeth have been reduced more rapidly than their earlier developing neighbors. Within each class, greater evolutionary reduction is also associated with greater phenotypic variability in present populations, and this greater variability appears to be due to a larger environmental component. Part of this component seems to result from local interactions between teeth developing in a confined space. The apparent paradox of the association of more rapid evolutionary change with greater non-genetic variability can be explained on a developmental basis which involves compensatory interactions between early and late developing teeth of the same class under the constant restricting pressure of a changing skeletal system.

Literature Cited

Bader, R. S. 1965. Heritability of dental characters in the house mouse. Evolution 19:378–384.

Bader, R. S., and W. H. Lehmann. 1965. Phenotypic and genotypic variation in odontometric traits of the house mouse. Amer. Midl. Natur. 74:28–38.

Bailit, H. L., S. J. DeWitt, and R. Leigh. 1968. The size and morphology of the Nasioi dentition. Amer. J. Phys. Anthrop. 28:271–288.

Bailit, H. L., P. L. Workman, J. D. Niswander, and C. J. MacLean. 1970. Dental asymmetry as an indicator of genetic and environmental conditions in human populations. Hum. Biol. 42:626–638.

Brace, C. L. 1967. Environment, tooth form and size in the Pleistocene. J. Dent. Res. 46: 809–816.

Dahlberg, A. A. 1945. The changing dentition of man. J. Amer. Dent. Assoc. 32:676–690.

Falconer, D. S. 1964. Introduction to Quantitative Genetics. Oliver & Boyd, Edinburgh.

Friedlander, J. S., and H. L. Bailit. 1969. Eruption times of the deciduous and permanent teeth of natives of Bougainville, territory of New Guinea: A study of racial variation. Hum. Biol. 41:51–65.

Garn, S. M., A. B. Lewis, and R. S. Kerewsky. 1965. Size interrelationships of the mesial and distal teeth. J. Dent. Res. 44:352–354.

Grewal, M. S. 1962. The development of an inherited tooth defect in the mouse. J. Embryol. Exp. Morph. 10:202–211.

Grüneberg, H. 1951. The genetics of a tooth defect in the mouse. Proc. Roy. Soc., B. 138: 437–451.

Hunter, W. S. 1959. The inheritance of mesiodistal tooth diameter in twins. Ph.D. Thesis. University of Michigan, Ann Arbor.

Lundström, A. 1948. Tooth Size and Occlusion in Twins. S. Karger, New York.

Orban, B. J. 1957. Oral Histology and Embryology. The C. V. Mosby Co., St. Louis.

Robinson, J. T. 1954. Prehominid dentition and hominid evolution. Evolution 8:324–334.

Sofaer, J. A. 1969. Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. J. Embryol. Exp. Morph. 22:181–205.

——. 1969. Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. J. Embryol. Exp. Morph. 22:207–227.

Sofaer, J. A., C. S. Chung, J. D. Niswander, and D. W. Runck. 1971. Developmental interaction, size and agenesis among permanent maxillary incisors. Hum. Biol. 43:36–45.

Van Valen, L. 1962. Growth fields in the dentition of *Peromyscus*. Evolution 16:272–278.

Reprinted from EVOLUTION, Vol. 27, No. 3, November 20, 1973
pp. 427–434
Made in United States of America

A MODEL RELATING DEVELOPMENTAL INTERACTION AND DIFFERENTIAL EVOLUTIONARY REDUCTION OF TOOTH SIZE

J. A. SOFAER
University of Cambridge, Department of Genetics, Milton Road, Cambridge, CB4 1XH, England

Received September 18, 1972

Reduction in size of the jaws during hominid evolution has been accompanied by an overall reduction of tooth size, but the degree of reduction varies from one tooth to another in a definite pattern. Within each morphological class (incisors, premolars and molars) the later a tooth develops the more it has been reduced. Similar relationships between developmental timing and degree of reduction may have been commonplace during the evolution of mammalian dentitions in general (Ziegler, 1971). However, later developing teeth tend to have lower heritabilities for tooth size than their earlier developing neighbours (Lundström, 1948; Hunter, 1959; Bader, 1965; Bader and Lehmann, 1965; Sofaer et al., 1971*a*). Thus there appears to be an association between lower heritability and more rapid evolutionary change. It is possible that this is the result of selection that has acted directly on the later developing teeth of each class causing a reduction of genetic variation. It is perhaps more logical to consider that the bulk of any selection acts on each morphological class as a whole rather than differentially on the individual teeth of which it is composed.

In the absence of direct differential selection on members of the same class the association between lower heritability and more rapid evolutionary change can be explained if it is assumed that some proportion of the reduction that has taken place in the dentition has been secondary to skeletal reduction, through selection for harmony between size of teeth and size of jaws. In such a system the primary reduction of jaw size might be expected to have resulted in restriction of the developing teeth, with consequent compensatory interaction between adjacent tooth germs due to competition for requirements necessary for growth. That is, if for some reason a tooth which developed early was larger than normal, then its later developing neighbour would have tended to be smaller than normal, and vice versa. There is evidence to suggest that this kind of compensatory interaction occurs in contemporary populations of both man and experimental animals (Grüneberg, 1951; Grewal, 1962; Van Valen, 1962; Gould and Garwood, 1969; Sofaer, 1969*a*; Sofaer et al., 1971*a*; Sofaer et al., 1971*b*).

In order to explain differential tooth reduction in terms of selection for harmony between tooth size and jaw size, it is necessary to show that genotypes with the potential to produce relatively large early developing teeth and small late developing teeth are favoured over those with the potential to produce relatively small early and large late teeth. A simple example of how compensatory interaction under conditions of local restriction can produce this result has already been proposed (Sofaer et al., 1971*a*). The purpose of the present paper is to put forward a generalised model and to discuss the patterns of variation that might be expected in contemporary populations if the model were valid.

THE MODEL

The assumptions underlying the model are as follows. Firstly, reduction in size of the teeth has been to some extent secondary to reduction in size of the jaws, through selection for harmony between tooth size

and jaw size. This selection is taken to act primarily on each morphological class as a whole rather than on the individual teeth of which it is composed. Secondly, compensatory interaction can occur between developing teeth, the later developing teeth tending to compensate for the combined deviations of their earlier developing neighbours from the norm; and thirdly, early developing teeth experience a relatively unrestricted local environment whereas later developing teeth tend to suffer most from the effects of skeletal restriction, simply because later developing teeth must make do with what remains of any local requirements that are necessary for growth.

If these assumptions are valid, it follows that, when size reduction was occurring, dental change lagged behind skeletal change and that the teeth were, on average, always genetically too large for the jaw in which they developed. However, the degree of restriction suffered by each tooth would have depended on the time at which it developed. The relative difference between the levels of local environmental restriction experienced by early and late developing teeth would have allowed more complete realisation of genetic size potential by the earlier developing teeth. If for the purpose of illustration it is assumed that the growth of early developing teeth was quite unrestricted, then early developing teeth would have grown to their full genetic potentials whereas later developing teeth would have tended always to be smaller than their genetic potentials. Thus, in a hypothetical class of two teeth the situation could be summarised in this way: $P_1 = G_1$, whereas $P_2 = G_2 - d$, where P_1, G_1, P_2 and G_2 are the phenotypes and genetic potentials of the earlier and later developing teeth respectively, and where d is the deviation due to local environmental restriction.

The magnitude of d clearly depends on at least two factors: the genetic potential of the later developing tooth, and the "space" available. (The use of the term "space" is perhaps an oversimplification, but it is meant to imply all the local requirements necessary for growth.) The greater the genetic potential of the later developing tooth the more it will be restricted in a given space, and the smaller the space the greater will be the restriction suffered by a tooth of a given potential. Thus d is proportional to G_2/S_2, where S_2 is the space available for the later developing tooth; but when $G_2 = S_2$, d should approximate to zero.

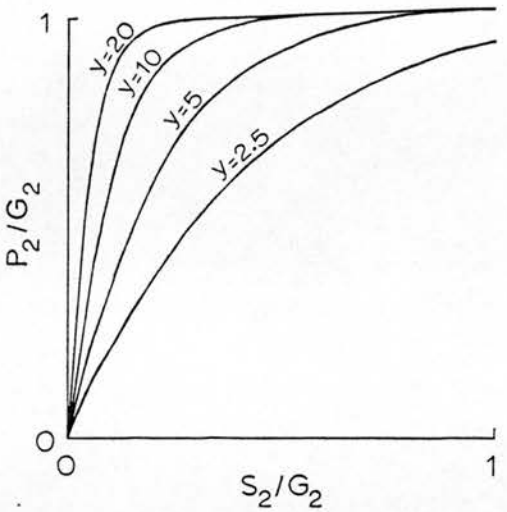


FIG. 1. The relationship between the phenotype of the later developing tooth (P_2) and the space available (S_2) in terms of the genetic potential of the later developing tooth (G_2), for different values of y, where $P_2 = G_2(1 - e^{-yS_2/G_2})$.

A relationship between P_2 and G_2 that has these qualities is:

$$P_2 = G_2\,(1 - e^{-yS_2/G_2})$$

where y is a factor that determines the rate of approach to $P_2 = G_2$ with increase in S_2. In this relationship: $d = G_2\, e^{-yS_2/G_2}$. Figure 1 illustrates the relationship between P_2/G_2 and S_2/G_2 for different values of y.

It has been pointed out above that in order to provide an explanation for the more rapid evolutionary reduction of the later developing teeth it must be shown that genotypes with the potential to produce large early and small late developing teeth were likely to have been favoured over genotypes with the potential to pro-

duce small early and large late teeth. That is, for a given combined genetic potential of early and late teeth under conditions of restriction, the combined phenotype of the large early and small late combination should be smaller than that of the small early and large late combination; or, put another way, $P_1 + P_2$ should decrease as $G_1 - G_2$ increases from negative to positive, with $G_1 + G_2$ constant and with $S < G_1 + G_2$, where S is the total space available for both teeth. When $S \geqq G_1 + G_2$, $P_1 + P_2$ should remain constant and approximately equal to $G_1 + G_2$.

Figure 2 illustrates the relationship between $(P_1 + P_2)/(G_1 + G_2)$ and $(G_1 - G_2)/(G_1 + G_2)$ for different levels of restriction and different values of y. It can be seen that the requirements necessary to explain differential tooth reduction in terms of developmental interaction and selection for harmony between tooth size and jaw size are satisfied. The model therefore provides a possible basis for interpreting the trend of evolution.

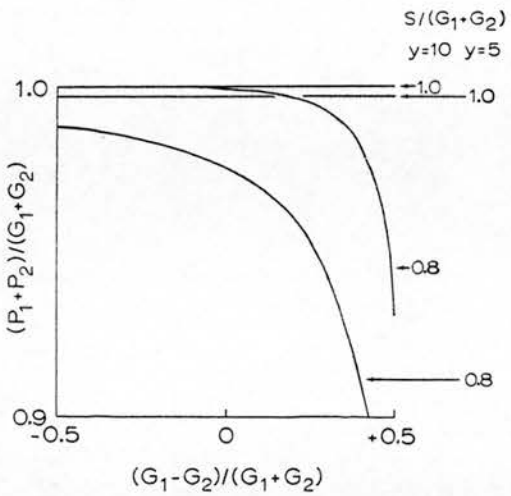


FIG. 2. The relationship between the combined phenotype of early and late developing teeth $(P_1 + P_2)$ and the difference between their genetic potentials $(G_1 - G_2)$ in terms of the constant sum of genetic potentials $(G_1 + G_2)$. The relationship is shown for two levels of restriction, where S is the space available for both teeth, with $S/(G_1 + G_2)$ equal to 1.0 (no restriction) and 0.8 (space available is 80% of combined potential), and for two different values of y. $P_1 = G_1$, $S_2 = S - P_1$, $P_2 = G_2(1 - e^{-yS_2/G_2})$. At zero on the horizontal axis early and late developing teeth have the same genetic potential. To the left of zero the potential of the late tooth is greater than that of the early tooth, and to the right of zero the potential of the late tooth is less than that of the early tooth.

A PREDICTION BASED ON THE MODEL

The validity of the model clearly cannot be tested in an evolutionary context. However, the model does imply particular patterns of variation among populations of contemporary individuals, and these patterns can be compared with suitable observations.

Patterns of variation predicted by the model were determined by computer simulation. Considerations were restricted to a hypothetical class composed of two teeth, one early and one late developing, in samples of 50 individuals from several hypothetical genetically homogeneous populations. The genetic potential of the early developing tooth was varied from one population to another, but the genetic potentials of the total space available (S) and of the late developing tooth (G_2) were kept constant $(S = 2G_2)$. For each of these hypothetical populations the mean phenotype of the early tooth was taken to be equal to the common genetic potential for the population, but each individual was assigned a phenotype (P_1) for each of his early teeth (on the right and left sides) which deviated from the mean value. The deviations were alotted randomly with respect to magnitude and direction, but over the whole of each population sample the size of the early developing tooth on each side was normally distributed with a standard deviation equal to five % of the mean. The deviations represented random developmental variation associated with a constant genetic potential.

The value of S_2 was varied by assigning values of S to each side of each individual at random from a normally distributed

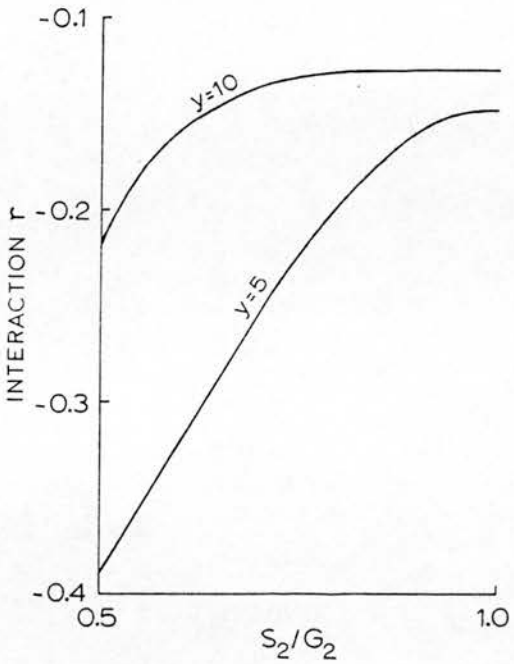


FIG. 3. The predicted relationship between the interaction correlation, r, and the level of space restriction suffered by the later developing tooth, S_2/G_2, for different values of y. G_1/G_2 is in the range 1 to 1.5, $S = 2G_2$, $P_2 = G_2(1 - e^{-yS_2/G_2})$.

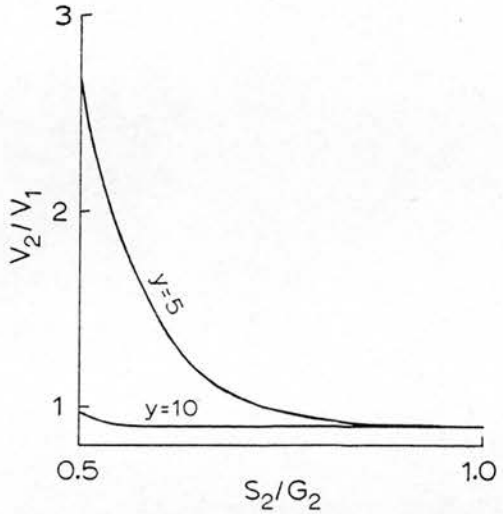


FIG. 4. The predicted relationship between the asymmetry variance ratio, V_2/V_1, and the level of space restriction suffered by the later developing tooth, S_2/G_2, for different values of y. G_1/G_2 is in the range 1 to 1.5, $S = 2G_2$, $P_2 = G_2(1 - e^{-yS_2/G_2})$.

hypothetical group of S values with a standard deviation equal to five % of the common mean for all populations. For each side of each individual S_2 was then equal to $S - P_1$.

The phenotype of the late developing tooth was calculated for each side of each individual from the relationship $P_2 = G_2(1 - e^{-yS_2/G_2})$ for different values of y. Each value of P_2 calculated in this way was regarded as a mean for the particular values of S_2 and y. Each actual value of P_2 assigned to each side of each individual was selected at random from a hypothetical group of P_2 values normally distributed around the calculated mean with a standard deviation equal to five % of G_2.

Having assigned phenotypes to early and late developing teeth on each side of each individual a number of variances and correlations between variables based on these phenotypes were calculated.

A quantity that has bearing on the model and that can be estimated easily in contemporary populations is the degree of asymmetry shown by corresponding teeth on the two sides of the jaw. This is usually expressed by the variable $(R - L)/(R + L)$ for each individual, where R and L are corresponding measurements on the right and left sides. This is the difference between sides relative to the absolute size of the measurements being made, allowing asymmetry in objects of different size to be compared directly. Two relevant statistics can be calculated using this variable.

The first statistic is the correlation between values of $(R - L)/(R + L)$ for two adjacent pairs of teeth. This estimates the nature of the interaction between adjacent teeth. If there is compensatory interaction, then, when $R - L$ for one tooth pair is positive, $R - L$ for the other will tend to be negative, producing a negative correlation. Conversely, a positive correlation means that, for both tooth pairs, teeth tend to be larger or smaller on the same side of the jaw. Figure 3 shows the predicted relation-

ship between the interaction correlation and the level of restriction for different values of y. The greater the restriction (S_2/G_2 smaller) the greater the negative correlation. The reason for this is clear on examination of Figure 1. For a given difference between sides in S_2, a low level of restriction will produce a smaller difference between sides in P_2 than a high level of restriction.

The second statistic of interest is the variance of $(R - L)/(R + L)$ for each tooth pair in each population. This is an estimate of the degree of asymmetry shown by each tooth pair in each population as a whole. The ratio of the asymmetry variance of one tooth pair to that of the other changes with the level of restriction, as shown in Figure 4. The greater the restriction (S_2/G_2 smaller) the greater the ratio V_2/V_1, where V_1 and V_2 are the asymmetry variances of the early and late developing teeth respectively. This occurs for the same reason as does the change in the interaction correlation.

It should be emphasised here that Figs. 3 and 4 are specific to the conditions built into the model. As far as real populations are concerned these Figures can only suggest a general pattern of possible relationships. There are presumably many factors other than those considered that have some effect on tooth size and that could perhaps modify the picture. For example, there is a tendency for the sizes of adjacent teeth on the same side of the jaw to be positively correlated, due to common local environmental influences during development. Thus there must be a balance between the reaction to common local environment on the one hand and the necessity for compensatory interaction imposed by space restriction on the other. It may in fact be possible to estimate the relative magnitude of these opposing influences (Van Valen, 1962; Gould and Garwood, 1971). It is therefore reasonable to suppose that for real populations the interaction correlation approaches a maximum at some positive value rather than at a level just below zero.

Evidence in support of the model might be forthcoming if the interaction correlation and the asymmetry variance ratio could be related to the level of space restriction in different populations. However, estimation of the level of restriction poses a problem since the "space" of relevance is that which is available at a particular phase of development, and measurements in adults may not provide a good indication of this. A possible solution is to relate the asymmetry variance ratio to the interaction correlation, without direct consideration of the level of restriction. Reference to Figs. 3 and 4 shows that the greater the level of compensatory interaction (greater negative correlation) the greater the asymmetry variance ratio. A simple test of trend would therefore be to rank a number of populations by interaction correlation and by asymmetry variance ratio and to perform a rank correlation between the two statistics. Aside from the model there does not appear to be any *a priori* reason why the predicted relationship should be found. On the contrary, it might be argued that greater asymmetry in the early tooth pair would be likely to induce greater negative correlation.

A Test of the Prediction

The predicted relationship between the asymmetry variance ratio and the interaction correlation was tested for twelve groups of mice, each group comprising around 25 individuals. Three of the groups were the inbred strains A/Fa and JU/Fa, and their F_1; two were composed of wild-type segregants from two stocks carrying different mutant alleles of the X-linked gene *tabby*; and the remaining seven groups comprised wild-type progeny of crosses of these stocks, and of backcrosses, to the two inbred strains (Sofaer, 1969*b*). The twelve groups therefore presented a variety of genetic constitutions.

The mouse normally has three molars in each quadrant. The basic configuration of the crown is established entirely prenatally for the first molar and largely prenatally

TABLE 1. *The genetic constitutions of the twelve groups*; the number of individuals of each group in which 1st and 2nd molars were measured on both sides,* N*; the interaction correlation,* r*; and the asymmetry variance ratio,* V_2/V_1. *All individuals were wild-type at the tabby locus.*

Genetic Constitution of Group				Upper Jaw					Lower Jaw				
					Mean ± S.E. of (R + L)/2					Mean ± S.E. of (R + L)/2			
A/Fa	JU/Fa	*Ta*^{f} stock background	*Ta*^{c} stock background	*N*	1st molar	2nd molar	*r*	V_2/V_1	*N*	1st molar	2nd molar	*r*	V_2/V_1
1				26	172.69 ± 0.65	91.88 ± 0.63	−.18	4.76	26	142.87 ± 0.35	87.81 ± 0.59	−.28	2.39
	1			23	176.43 ± 0.60	102.04 ± 0.40	.39	1.59	23	144.59 ± 0.40	94.78 ± 0.37	.13	0.78
½	½			23	180.26 ± 0.58	99.70 ± 0.45	−.26	4.16	24	145.02 ± 0.32	92.98 ± 0.38	−.01	3.84
		1		22	181.82 ± 1.36	100.18 ± 0.48	.03	1.14	22	148.36 ± 1.02	95.89 ± 0.73	.01	2.18
	½	½		22	184.75 ± 0.83	102.73 ± 0.51	−.65	4.47	20	151.23 ± 0.57	98.50 ± 0.56	−.35	4.59
½		½		22	183.77 ± 0.93	99.95 ± 0.86	−.07	3.92	22	147.23 ± 0.87	94.70 ± 0.92	−.20	1.25
¾		¼		22	179.64 ± 0.77	100.39 ± 0.60	.16	5.36	21	145.12 ± 0.58	93.93 ± 0.66	.05	3.03
			1	22	189.34 ± 1.97	105.91 ± 1.30	−.12	1.46	22	151.00 ± 1.26	99.84 ± 1.08	−.24	1.43
	½		½	20	181.30 ± 1.01	100.87 ± 0.52	−.25	2.67	22	148.95 ± 0.91	96.20 ± 0.69	−.47	2.35
	¾		¼	22	179.23 ± 0.83	99.25 ± 0.50	.12	2.17	22	147.91 ± 0.42	95.34 ± 0.57	−.59	0.94
½			½	22	183.25 ± 0.78	100.64 ± 0.66	.01	1.80	22	150.48 ± 0.58	96.11 ± 0.59	−.19	1.32
¾			¼	22	182.77 ± 1.06	99.16 ± 0.66	.11	6.59	22	148.23 ± 0.72	93.50 ± 0.68	.40	0.61

* Details of the crosses between strains are given in Sofaer 1969b.

for the second molar. For both the first and second molars the time taken from the appearance of a tooth bud to the onset of dentine formation is approximately eight days, and about two days separate equivalent stages of differentiation in the two teeth (Gaunt, 1955; Cohn, 1957; Sofaer, 1969*a*). The third molar, on the other hand, develops entirely postnatally and is subject to the environmental rigours of the immediate postnatal period (Grüneberg, 1951; Searle, 1954; Tenczar and Bader, 1966). Since the model assumes that the general environment during tooth development remains constant, it is appropriate to consider only the first and second molars in a test of any prediction based on the model.

The anteroposterior lengths of upper and lower first and second molars of the twelve groups were measured in a projection microscope by projecting a magnified silhouette (×100) of each tooth to be measured onto a graduated screen. Each measurement, which was made to the nearest $\frac{1}{100}$ mm, was the maximum anteroposterior diameter of the crown parallel to the occlusal plane.

Tooth size, the interaction correlation and the asymmetry variance ratio are listed for upper and lower first and second molars in Table 1. The Spearman and Kendall rank correlations between the interaction correlation and the asymmetry variance ratio over the twelve groups are $r_s = -.15$ and $\tau = -.15$ for upper first and second molars, and $r_s = -.29$ and $\tau = -.21$ for lower first and second molars. There is therefore a slight though non-significant tendency for greater compensatory interaction to be associated with greater asymmetry of the later developing tooth in each jaw.

Since there is no indication of the precise nature of the relationship between the interaction correlation and asymmetry variance ratio it seems that a ranking test is the best that can be applied to the data. However, assuming the model to be valid, there are at least two reasons why conventional significance levels may be misleading. Firstly, Figs. 3 and 4 show that the relationship between the two statistics is likely to be non-linear; and secondly, it is possible that the underlying pattern of the relationship is not entirely the same for all genotype groups. Both of these are complications that would tend to reduce the level of correlation. Considered in this light, the results, together with the ability of the mechanisms discussed here to account for the trend of evolution, indicate that the model may be of some value when considering the basis for evolutionary changes of tooth size.

Summary

Reduction in size of the jaws during hominid evolution has been accompanied by a general reduction of tooth size, but within each morphological class the later a tooth develops the more it has been reduced. This pattern of differential reduction can be explained by assuming that a proportion of the reduction that has taken place in the dentition has been secondary to skeletal reduction, through selection for harmony between size of teeth and size of jaws. Primary reduction of jaw size implies that the teeth were always genetically too large for the jaw in which they developed, and under such conditions of restriction were likely to have shown compensatory interaction due to competition for requirements necessary for growth.

In order to explain differential reduction of tooth size in terms of selection for harmony between size of teeth and size of jaws it is necessary to show that genotypes with the potential to produce relatively large early and small late developing teeth were likely to have been favoured over genotypes with the potential to produce relatively small early and large late developing teeth. That is, it must be shown that the genotypically large early and small late combination results in a smaller combined phenotype, which is presumably better suited to a reduced jaw. A model that embodies these features has been proposed. The model predicts that among populations

of contemporary individuals greater compensatory interaction is associated with greater asymmetry of the late relative to the early developing teeth within a morphological class. A test of this prediction in twelve genetically different samples of mice showed a slight though non-significant tendency in accordance with this prediction for the relationship between first and second molars in both the upper and lower jaws.

Acknowledgments

Financial support in the form of a Nuffield Foundation Dental Research Fellowship and a Research Project Grant from the Medical Research Council is gratefully acknowledged. Mrs. Christa Lucas made the tooth measurements.

Literature Cited

Bader, R. S. 1965. Heritability of dental characters in the house mouse. Evolution 19:378–384.

Bader, R. S., and W. H. Lehmann. 1965. Phenotypic and genotypic variation in odontometric traits of the house mouse. Amer. Midl. Natur. 74:28–38.

Cohn, S. A. 1957. Development of the molar teeth in the albino mouse. Amer. J. Anat. 101:295–310.

Gaunt, W. A. 1955. The development of the molar pattern of the mouse (*Mus musculus*). Acta Anat. 24:249–268.

Gould, S. J., and R. A. Garwood. 1969. Levels of integration in mammalian dentitions: an analysis of correlations in *Nesophontes micrus* (Insectivora) and *Oryzomys couesi* (Rodentia). Evolution 23:276–300.

Grewal, M. S. 1962. The development of an inherited tooth defect in the mouse. J. Embryol. Exp. Morph. 10:202–211.

Grüneberg, H. 1951. The genetics of a tooth defect in the mouse. Proc. Roy. Soc. B. 138: 437–451.

Hunter, W. S. 1959. The inheritance of mesiodistal tooth diameter in twins. Ph.D. Thesis. University of Michigan, Ann Arbor.

Lundström, A. 1948. Tooth size and occlusion in twins. S. Karger, New York.

Searle, A. G. 1954. Genetical studies on the skeleton of the mouse. XI. The influence of diet on variation within pure lines. J. Genet. 52:413–424.

Sofaer, J. A. 1969*a*. Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. J. Embryol. Exp. Morph. 22:181–205.

——. 1969*b*. Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. J. Embryol. Exp. Morph. 22:207–227.

Sofaer, J. A., H. L. Bailit, and C. J. MacLean. 1971*a*. A developmental basis for differential tooth reduction during hominid evolution. Evolution 25:509–517.

Sofaer, J. A., C. S. Chung, J. D. Niswander, and D. W. Runck. 1971*b*. Developmental interaction, size and agenesis among permanent maxillary incisors. Hum. Biol. 43:36–45.

Tenczar, P., and R. S. Bader. 1966. Maternal effect in dental traits of the house mouse. Science 152:1398–1400.

Van Valen, L. 1962. Growth fields in the dentition of *Peromyscus*. Evolution 16:272–278.

Ziegler, A. C. 1971. A theory of the evolution of therian dental formulas and replacement patterns. Quart. Rev. Biol. 46:226–249.

DENTAL MORPHOLOGICAL VARIATION AND POPULATION CLASSIFICATION

Dental morphology is used traditionally for phylogenetic classification and has also been applied to comparisons between contemporary populations. However, little is known of the genetic contribution to the observed morphological variation, so the biological significance of such comparisons has been a matter for speculation. The three papers in this section have a bearing on this problem. In the mouse, the expression of a supernumerary cusp varies from one inbred strain to another; in man, the later developing teeth within a morphological class exhibit a lower additive component of genetic variation; and lastly, in a comparison of contemporary human populations, moderately good correspondence is found between the degree of population difference based on known genetic variants and that based on tooth morphology alone.

Archs oral Biol. Vol. 14, pp. 1213–1223, 1969. Pergamon Press. Printed in Gt. Britain.

THE GENETICS AND EXPRESSION OF A DENTAL MORPHOLOGICAL VARIANT IN THE MOUSE

J. A. SOFAER*

The Institute of Animal Genetics, West Mains Road, Edinburgh, Scotland

Summary—A supernumerary cusp on the lower first molar of the mouse is described. By means of appropriate crosses of affected animals to different inbred strains it is shown that the cusp behaved as a quasi-continuous variable and that its presence or absence was not controlled by a single gene. Limitations involved in the genetic analysis of morphological characters not showing discrete or metric variation are discussed.

INTRODUCTION

SPECIFIC morphological variants in the dentition of the mouse have been shown to be characteristic of different inbred strains (GRÜNEBERG, 1965). There is therefore reason to believe that they are to some extent under genetic control. The variant considered here, a supernumerary cusp on the lower first molar, has not been described previously. The cusp varied in penetrance and expressivity and, as such, showed quasi-continuous rather than discrete variation. When investigating the genetic control of characters not showing discrete variation, appropriate tests are required to demonstrate a single gene (WEBER, 1959; BLOOM and FALCONER, 1964). In the absence of evidence for a single gene the implication is that the genetic component of variation of the character is due to segregation at more than one locus. Under certain circumstances it may be possible to estimate experimentally, or infer from a response to selection, the actual number of loci involved in such a multifactorial situation (WEHRHAHN and ALLARD, 1965; THODAY, GIBSON and SPICKETT, 1964), and even the relative magnitude of each gene's effect (WEHRHAHN and ALLARD, 1965). However, detailed analysis of this sort would not have been possible here because of the non-metric nature of the character. Investigation of the genetic control of the supernumerary cusp was therefore restricted to discriminating between single gene and multifactorial inheritance. In addition, the pattern of its variable expression was considered in terms of the criteria of quasi-continuous variation (GRÜNEBERG, 1952).

MATERIAL

Variation in the form of mouse molars is largely composed of small differences of relative size and position of the normal complement of cusps, but may include a difference of cusp number. The normal crown of the lower first molar is composed of seven cusps. Numbered from anterior to posterior there are three buccal, B1, B2, and

* Present address: National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland, 20014, U.S.A.

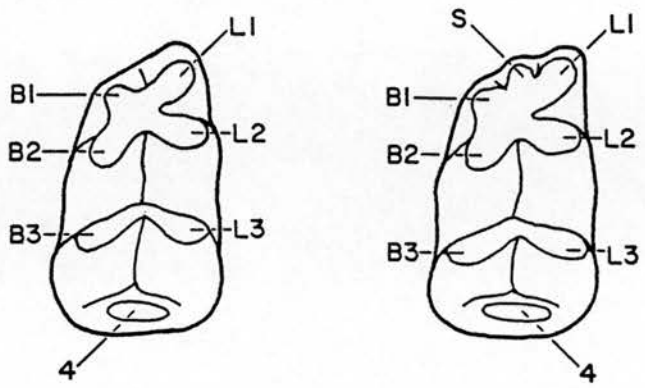


FIG. 1. Diagrams of occlusal surfaces of normal (left) and Tuck (right) lower left first molars.

B3; three lingual, L1, L2, and L3; and a single central posterior cusp, 4. A supernumerary cusp, S, between B1 and L1 was found to occur with high frequency among animals of the Tuck No. 1 strain (Fig. 1). At its largest the cusp was comparable in size and form with its neighbours, but more usually it consisted of a projection of variable size, either from the groove between B1 and L1, or from the antero-buccal surface of L1. In very many cases the impression was given of different degrees of division of L1 into two daughter cusps. In a few cases the cusp was restricted to the base of a widened groove between B1 and L1. Normal and Tuck teeth are shown in Fig. 2.

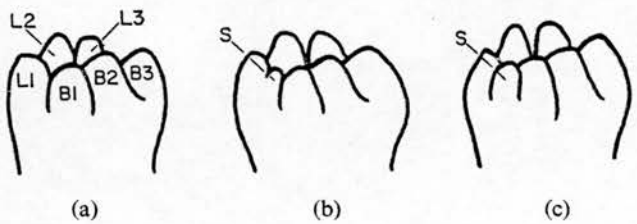


FIG. 2. Antero-buccal views of lower left first molars. (a) Normal (b) Small supernumerary cusp, and (c) Large supernumerary cusp.

The Tuck No. 1 strain has been maintained by random mating within a closed colony for 20 years. Tuck animals are therefore not highly inbred but can be regarded as being genetically similar. Three matings were obtained from Tuck's Mousery, Rayleigh, Essex, and the offspring of these matings were subsequently mated at random to provide material for the experiment. Eighty out of 89 Tuck animals showed a supernumerary cusp, some on one side and others on both. Such phenotypic variation does not necessarily imply genetic heterogeneity (SEARLE, 1954a), but in order to reduce the possible effects of any genetic variation only the most severely affected Tuck animals were used for mating.

Four inbred strains were used in crosses with Tuck animals. These were strains *A*, *C57*, *JU*, and *CBA*, which have been maintained by brother × sister matings at the Institute of Animal Genetics, Edinburgh, for between 40 and 50 generations. Animals of the same strain can therefore be regarded as being genetically identical. Lower first molars of a number of these inbred mice were examined. Six out of 87 *C57* animals showed unilateral slight but definite grooving of the antero-buccal surface of L1 toward its tip, similar to that seen in the most mildly affected Tuck animals (Fig. 2b). The remaining *C57* animals, and all those of other three strains, were entirely normal in this respect. It should be mentioned that cusps B1 and L1 of *C57* lower first molars are always much less well separated than they are in the other three strains. This minimal separation of B1 and L1 in *C57* mice has already been noted by GRÜNEBERG (1965). Cusps B1 and L1 of the few unaffected Tuck teeth were well separated.

METHOD

(*a*) *Scoring and examination*

In the genetic analysis the cusp was treated as an all-or-none character. However, as it varied in size, some attempt was made to score the degree to which animals were affected, so that expressivity as well as penetrance could be considered. Animals were scored 0 for no cusp, 1 for a small cusp, and 2 for a large cusp. Each side was scored separately, so that the maximum possible total score was 4. The results of the crosses are expressed as histograms based on this method of scoring.

Animals required for mating were examined under anaesthesia produced by an intraperitoneal injection of Nembutal (0·1 ml of a 0·9 per cent solution per 10 g body wt.). Scoring required a special light source as the first molars are too far back from the small opening of the oral cavity to be seen with conventional illumination. A dissecting microscope was used with a glass slide held at 45° just below the objective lens. A horizontal light beam was directed at the slide which reflected light down along the optical axis of the lens. At the same time the slide allowed an image of sufficient intensity to pass back through it unreflected and to be observed through the microscope. A pair of adapted tweezers served as a mouth prop, and a small funnel-shaped instrument was constructed to act as a retractor for the tongue and lips through which the observer could see. Animals not required for mating were sacrificed and dissected prior to examination.

Tuck animals and the progeny of the crosses were examined between weaning at 3 weeks and mating at 6 weeks of age. Some of the inbred mice were a little older, but not enough to allow wear to affect scoring.

(*b*) *Experimental design*

When affected Tuck animals were crossed to the four inbred strains there was a striking difference between the F_1 of the *C57* cross, where the character behaved as almost completely dominant, and those of the other three, where it behaved as almost completely recessive. This variable expression of the character on different genetic backgrounds enabled two parallel sets of crosses to be carried out, one of which would have been able to demonstrate a dominant gene, and the other of which would have

been able to demonstrate a recessive gene. F_1 animals of the *C57* cross were backcrossed to *C57*, and then a second backcross was made to *C57*. F_1 animals of the *JU* cross, taken as being representative of the recessive situation, were backcrossed to affected Tuck animals, and then a second backcross was made to Tuck.

When dealing with a character showing variable penetrance and expressivity, meaningful conclusions can only be drawn if an underlying scale of continuous variation is assumed to exist. Such a scale would be a measure of some attribute immediately related to the development of the character and all individuals below a threshold value would be normal and all those above it affected. The more the value exceeded the threshold the more severely would the individual be affected. On this basis, the theory behind the system of crosses is illustrated in Fig. 3.

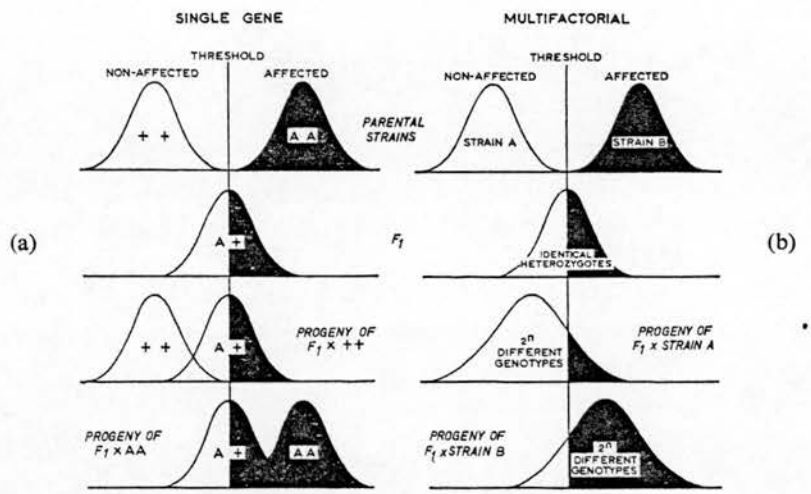


FIG. 3. The theoretical distributions of two parental strains, their F_1, and progeny of backcrosses of the F_1 to the two parental strains. (a) For a character controlled by a single gene with intermediate dominance. (b) For a character under multifactorial control. Based on the quasi-continuous variation model.

In the single gene case the two strains differ in respect of the gene A and one is all affected and one all non-affected. F_1 individuals are all identical heterozygotes with some above and some below the threshold. In the real case the F_1s were not exactly intermediate but high in the *C57* cross and low in the others. Backcrossing the F_1 produces two distributions, one identical to the F_1 and one identical to the parental strain to which it was crossed. The proportion of affected individuals amongst the first backcross progeny is therefore intermediate between the F_1 and the parental strain to which it was crossed. In the case of multifactorial inheritance, F_1 individuals are all identical heterozygotes with some above and some below the threshold, just as in the single gene case. But, unlike the single gene case, backcrossing produces a single distribution of greatly increased variance, composed of individuals of 2^n different genotypes, where n is the number of loci which are different between the two

parental strains. The progeny of the F_1 backcrossed to the unaffected parent form a distribution whose mean is shifted down from the threshold, and the progeny of the F_1 backcrossed to the affected parent form a distribution whose mean is shifted up from the threshold. The outcome, on an all-or-none basis, is just as in the single gene case. The proportion of affected individuals amongst the first backcross progeny is intermediate between the F_1 and the parental strain to which it was crossed. The two alternatives, single gene and multifactorial inheritance, are therefore indistinguishable at this stage. However, if there was a reliable scale composed of a sufficient number of subdivisions with which to classify affected individuals, the shape of the distribution of progeny of the first backcross to the affected strain would be an indication of which of the two alternatives applied.

The critical test to distinguish between single gene and multifactorial inheritance is made by a second backcross, where first backcross progeny are taken at random to be used as partners in mating to the original parental stocks. The genotypes of first backcross progeny are then reflected in the families they produce, and the families can be scored on the basis of the proportion of affected individuals they contain. The behaviour of the two alternative situations in a second backcross is illustrated in Fig. 4. A single gene is expected to produce a bimodal distribution of second backcross

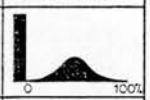
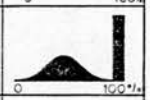
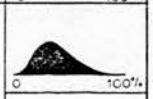
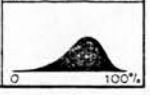
		1st BACKCROSS PROGENY	2nd BACKCROSS TO	TYPES of MATING	PROPN. AFFECTED INDIVIDUALS	DISTRIBUTION of FAMILY SCORES
SINGLE GENE	DOMINANT	½ ++	+ +	++ x ++	0%	
		½ A+		A+ x ++	50%	
	RECESSIVE	½ aa	a a	aa x aa	100%	
		½ a+		a+ x aa	50%	
MULTIFACTORIAL		2^n DIFFERENT GENOTYPES	NON-AFFECTED PARENT	MAXIMUM NUMBER OF 2^n	WIDE VARIATION BETWEEN FAMILIES	
			AFFECTED PARENT			

FIG. 4. The behaviour of single gene and multifactorial inheritance in a second backcross.

family scores, as there are two distinct genotypes of first backcross progeny which are used as parents. Multifactorial inheritance is expected to produce a unimodal distribution, as there are many different genotypes amongst the first backcross progeny.

RESULTS

The matings made and numbers of progeny produced are shown in Table 1. The incidence of affected individuals amongst the parental strains and the progeny of the crosses, according to the scoring method described, is shown in Fig. 5.

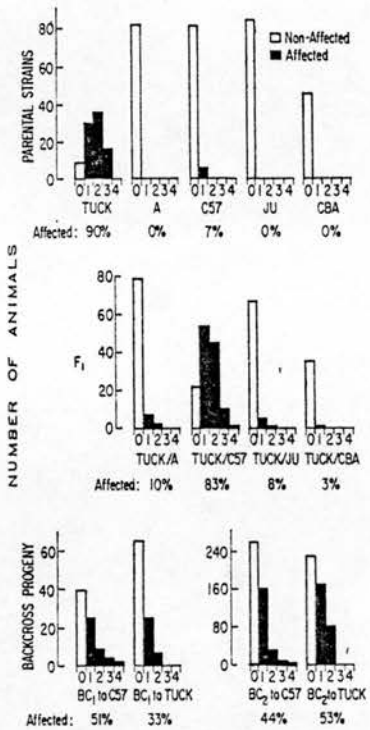


FIG. 5. The incidence of affected individuals amongst the parental strains and progeny of the crosses, according to the scoring method described in the text.

(a) Genetics

Comparison of the F_1 groups shows that the character was almost completely dominant on the *C57* background and almost completely recessive on the other three. As expected, the first backcross, both to *C57* and to Tuck, produced progeny amongst which the proportion of affected individuals was intermediate between the F_1 and the parental strain to which it was crossed. The scoring method was not fine enough to indicate the shapes of the distributions. The results at this stage are therefore equally compatible with single gene and multifactorial inheritance.

The results of the second backcross treated in the same way are similarly compatible with both forms of inheritance. The proportion of affected individuals in each case was intermediate between that of the first backcross group and the parental strain to which it was crossed. If, however, the second backcross results are plotted as distributions of family scores, the presence of a major gene should be detectable as a bimodality. Figure 6 shows the distributions of second backcross family scores. There was no definite trend towards bimodality in either cross, which indicates that no single gene was responsible for the presence of the cusp.

TABLE 1. MATINGS MADE AND THE NUMBERS OF PROGENY PRODUCED

Cross	♀ Parent	♂ Parent	Code	Number of matings	Number of progeny
Tuck × *A*	Tuck	*A*	TA	4	87
Tuck × *C57*	Tuck	*C57*	TCa	4	63
	C57	Tuck	TCb	8	68
Tuck × *JU*	*JU*	Tuck	TJa	3	57
	Tuck	*JU*	TJb	1	15
Tuck × *CBA*	*CBA*	Tuck	TCBA	4	36
BC_1 to *C57*	$C57F_1$	*C57*	TCC_1a	5	47
	C57	$C57F_1$	TCC_1b	5	32
BC_1 to Tuck	JUF_1	Tuck	TJT_1a	5	56
	Tuck	JUF_1	TJT_1b	4	41
BC_2 to *C57*	$C57BC_1$	*C57*	TCC_2a	11	193
	C57	$C57BC_1$	TCC_2b	13	266
BC_2 to Tuck	Tuck BC_1	Tuck	TJT_2a	13	253
	Tuck	Tuck BC_1	TJT_2b	12	228

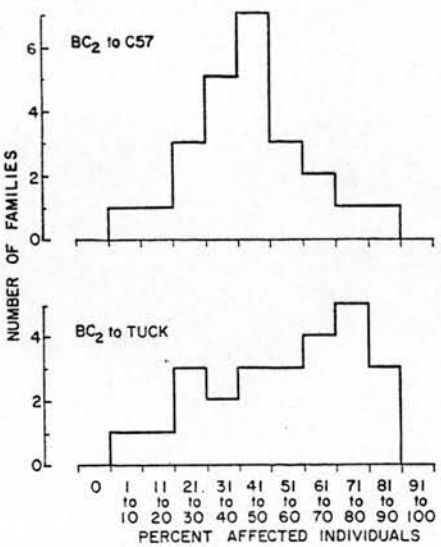


FIG. 6. Distributions of second backcross family scores.

Family size	12	13	14	15	16	17	18	19	20	21	22	23	24	25
No. of *C57* BC_2 families	1	0	2	0	1	5	1	3	2	3	2	1	2	1
No. of Tuck BC_2 families	0	0	0	0	4	5	4	1	3	0	5	2	0	1

(b) Pattern of variation

The proportions of affected individuals in each of the crosses conformed with what would be expected of an underlying scale of continuous variation. The group distributions of the two sets of crosses moved in opposite directions with successive backcrossing. Further evidence for such an underlying scale was obtained by examining the distributions of animals between classes within each affected group. This will now be described.

If an underlying scale of continuous variation does exist, then the greater the proportion of animals affected in any particular group the more severely will they be affected on average. This relationship between penetrance and expressivity was tested. Animals were scored in five classes: 0, 1, 2, 3 and 4. Zero must represent the threshold, so, if all classes above zero are to be of equal size, 1, 2, 3 and 4 must be the upper limits of each class. The midpoints of the four classes are then 0·5, 1·5, 2·5 and 3·5. It was from these midpoints that the observed mean score of affected animals in each group (observed MSA) was calculated.

Given the proportion of affected individuals in any one group, and assuming that the group is normally distributed, two values can be read from tables. These are, x, the distance of the threshold from the group mean, and a, the distance of the mean of affected individuals from the group mean. They are both in terms of σ, the standard deviation of the group (FALCONER, 1965). Thus, if σ is known, purely theoretical expected MSA values relative to the constant threshold (that is a-x), can be calculated for distributions which have different positions on the underlying scale.

Strict comparison between groups can only be made if the σ of each group is known. Accordingly, estimates of σ in terms of a constant, the threshold interval, were made (FALCONER, 1964). For this purpose animals were divided into three classes; those scored as 0, those scored as 1, and those scored as 2 and above. The values for σ were then calculated in terms of the interval between the 0–1 and 1–2 thresholds. Once σ for each group had been estimated the expected MSA values could all be expressed in terms of the common threshold interval as $\sigma\ (a - x)$.

The MSA expectation, based on the quasi-continuous variation model of a normal distribution moving across a fixed threshold, embodies an increase of MSA with percentage affected; that is, an increase of expressivity with penetrance. A positive correlation between expected MSA and observed MSA values would therefore demonstrate that such a relationship exists in the material under study.

Table 2 shows the particulars of nine groups considered. The *A*, *JU*, and *CBA* F_1 groups were omitted from the calculation of σ and MSA as they had respectively only 2, 1, and 0 animals in the upper class. Values for the *CBA* F_1 group could therefore not be calculated, and for the other two groups were unlikely to have been reliable.

Figure 7 shows the relationship between expected MSA and observed MSA. There was a very high positive correlation ($r = 0{\cdot}98$) and the regression ($b = 0{\cdot}86$) was not significantly different from unity. However, all the observed values were a little higher than would have been expected on the basis of complete colinearity of the underlying scale of continuous variation and the subjective scale of measurement used to score the phenotype. Nevertheless, the result does indicate that there was an increase

TABLE 2. THE PARTICULARS OF NINE GROUPS, FOR SIX OF WHICH σ AND EXPECTED AND OBSERVED MSA (MEAN SCORE OF AFFECTED INDIVIDUALS) WERE CALCULATED

Group code	Number scored in each category			% affected	σ	Expected MSA	Observed MSA
	0	1	2 and above				
Tuck	9	29	51	90	0·90	1·33	1·34
TA	78	7	2	10	—	—	—
TC a & b	22	53	56	83	0·88	1·10	1·12
TJ a & b	66	5	1	8	—	—	—
TCBA	35	1	0	3	—	—	—
TCC_1 a & b	39	25	15	51	1·17	0·94	1·08
TJT_1 a & b	65	25	7	33	0·97	0·64	0·72
TCC_2 a & b	258	158	43	44	0·84	0·63	0·77
TJT_2 a & b	227	167	87	53	1·01	0·84	0·86

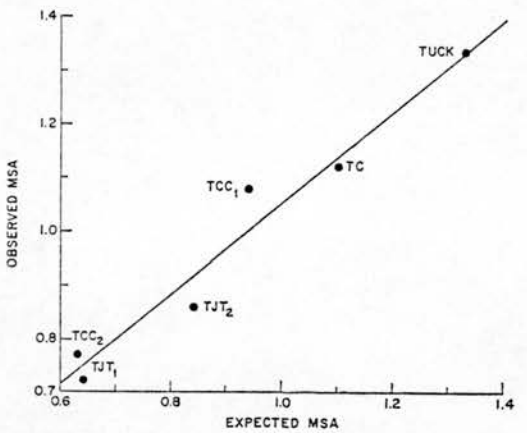


FIG. 7. The relationship between expected and observed MSA (mean score of affected individuals).

of expressivity with penetrance and is therefore consistent with the model of quasi-continuous variation.

Reciprocal crosses were then considered separately. Values for σ and M ($M = \sigma x$), the distance of the distribution mean from the 0–1 threshold in threshold units, were calculated for each reciprocal group. Reciprocal crosses of the *C57* groups showed a constant relationship in respect of both σ and M. The calculated values of both were lower for the progeny of *C57* mothers. No such relationship was apparent between reciprocals of the *JU* crosses. Reciprocal groups were then compared by a chi-square test using the actual numbers of animals scored in the three classes 0, 1 and 2 and above. The sexes were similarly compared, males of both reciprocal groups with females of both reciprocal groups within each cross. No differences were found between reciprocals of the *JU* crosses, but there were differences between reciprocals of two

of the three *C57* crosses ($P<0{\cdot}05$ and $P<0{\cdot}02$). Details of the reciprocals of the *C57* crosses are shown in Table 3. There was no difference between the sexes in any cross.

TABLE 3. DETAILS OF THE RECIPROCALS OF THE *C57* CROSSES. *M* IS THE MEAN OF EACH GROUP RELATIVE TO THE 0–1 THRESHOLD EXPRESSED IN THRESHOLD UNITS

Group code	Numbers scored in each category			χ^2	σ	M
	0	1	2 and above			
TCa	10	21	32	$\chi^2_2 = 3{\cdot}45$	1·03	+0·03
TCb	12	32	24	($P>0{\cdot}1$)	0·76	−0·29
TCC_1a	22	12	13	$\chi^2_2 = 6{\cdot}19$	1·52	−0·89
TCC_1b	17	13	2	($P<0{\cdot}05$)	0·68	−1·06
TCC_2a	105	61	27	$\chi^2_2 = 8{\cdot}51$	1·02	−1·10
TCC_2b	153	97	16	($P<0{\cdot}02$)	0·74	−1·15

If the difference between reciprocals were due to sex-linkage, consistent differences of a similar magnitude would be expected between the sexes. As these were not found, the difference between reciprocals, if it was a real one, must be attributed to a maternal effect. The difference between reciprocal F_1 groups was not significant, whereas the difference between reciprocal first and second backcross groups was. This could be related to the fact that both F_1 groups had parental strain mothers, whereas, in each backcross, one group had a *C57* mother and its reciprocal had a hybrid mother.

The three groups of progeny of *C57* mothers had lower means than those of Tuck and hybrid mothers. The animals expected to be the more vigorous mothers therefore produced more abnormal offspring. This seems to be at variance with previous findings (GRÜNEBERG, 1951; SEARLE, 1954b; DEOL and TRUSLOVE, 1957). However, the present abnormality is an addition to rather than a subtraction from the normal, and as such may only be expressed under optimum conditions. The three groups of progeny of *C57* mothers also had lower variances than their reciprocals. This could be explained in terms of narrower canalization of the normal phenotype.

CONCLUSIONS

There is little doubt that no single gene is responsible for the presence of this supernumerary cusp. The variability of expression must therefore have been due to segregation at more than one locus. This variability of expression appeared to fulfil three criteria of quasi-continuous variation: there was a marked difference between F_1 groups when affected animals were crossed to different inbred strains; increased penetrance was accompanied by increased expressivity; and there was some evidence for differences between reciprocal crosses. The behaviour of this part of a tooth is therefore analagous to the behaviour of whole third molars (GRÜNEBERG, 1952).

However, there is a difficulty involved in the analysis of a morphological character not showing discrete variation which does not apply to a metric character like third molar size. Although it may be obvious that the size of the morphological character varies continuously, precise measurement may not be possible. A rather arbitrary, subjective, and limited classification of expression, such as that used here, may have to be adopted. This then imposes limitations on the amount of information that can be derived, even from an appropriately designed experiment.

Acknowledgements—I am grateful to Professor H. GRÜNEBERG for suggesting a study of the Tuck No. 1 strain, to Professor D. S. FALCONER for his interest and valuable advice during the work, to Professor C. H. WADDINGTON for laboratory facilities, and to the Nuffield Foundation for financial support.

Résumé—Une cuspide surnuméraire de la première molaire inférieure de la souris est décrite. En croisant les animaux atteints à diverses souches, il apparait que cette cuspide se comporte comme une variable pratiquement continue et que sa présence ou son absence n'est pas sous le contrôle d'un seul gêne. Les limites de l'analyse génétique des caractères morphologiques, ne permettant pas de démontrer des variations discrètes ou métriques, sont envisagées.

Zusammenfassung—Es wird ein überzähliger Höcker am unteren ersten Molaren der Maus beschrieben. Durch Kreuzung der mit dieser Eigenart behafteten Tiere mit verschiedenen Inzuchtstämmen wird gezeigt, daß sich der Höcker wie eine quasi-kontinuierliche Variable verhält und daß dessen Vorhandensein oder Abwesenheit nicht durch ein einzelnes Gen kontrolliert wird. Die Grenzen der genetischen Analyse morphologischer Erscheinungsbilder, die weder diskrete noch metrische Variationen aufweisen, werden diskutiert.

REFERENCES

BLOOM, J. L. and FALCONER, D. S. 1964. A gene with major effect on susceptibility to induced lung tumors in mice. *J. natn. Cancer Inst.* **33**, 607–618.

DEOL, M. S. and TRUSLOVE, G. M. 1957. Genetical studies on the skeleton of the mouse. XX. Maternal physiology and variation in the skeleton of C57BL mice. *J. Genet.* **55**, 288–312.

FALCONER, D. S. 1964. *Introduction to Quantitative Genetics.* Chapter 18. Oliver & Boyd, Edinburgh.

FALCONER, D. S. 1965. The inheritance of liability to certain diseases, estimated from the incidence among relatives. *Ann. hum. Genet.* **29**, 51–76.

GRÜNEBERG, H. 1951. The genetics of a tooth defect in the mouse. *Proc. R. Soc.* B **138**, 437–451.

GRÜNEBERG, H. 1952. Genetical studies on the skeleton of the mouse. IV. Quasi-continuous variations. *J. Genet.* **51**, 95–114.

GRÜNEBERG, H. 1965. Genes and genotypes affecting the teeth of the mouse. *J. Embryol. exp. Morph.* **14**, 137–159.

SEARLE, A. G. 1954a. Genetical studies on the skeleton of the mouse. IX. Causes of skeletal variation within pure lines. *J. Genet.* **52**, 68–102.

SEARLE, A. G. 1954b. Genetical studies on the skeleton of the mouse. XI. The influence of diet on variation within pure lines. *J. Genet.* **52**, 413–424.

THODAY, J. M., GIBSON, J. B. and SPICKETT, S. G. 1964. Regular responses to selection. 2. Recombination and accelerated response. *Genet. Res.* **5**, 1–19.

WEBER, E. 1959. The genetical analysis of characters with continuous variability on a Mendelian basis. I. Monohybrid segregation. *Genetics, Princeton* **44**, 1131–1139.

WEHRHAHN, C. and ALLARD, R. W. 1965. The detection and measurement of the effects of individual genes involved in the inheritance of a quantitative character in wheat. *Genetics, Princeton* **51**, 109–119.

Archs oral Biol. Vol. 17, pp. 811–816, 1972. Pergamon Press. Printed in Great Britain.

HEREDITY AND MORPHOLOGICAL VARIATION IN EARLY AND LATE DEVELOPING HUMAN TEETH OF THE SAME MORPHOLOGICAL CLASS

J. A. SOFAER* and C. J. MACLEAN

Human Genetics Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20014, U.S.A.

and

H. L. BAILIT

University of Connecticut Health Center, Farmington, CT 06032, U.S.A.

Summary—Analysis of the resemblance between relatives from two Melanesian populations indicated that, for each of five morphological characters scored on an earlier and a later developing member of a tooth class, the later developing tooth showed a smaller component of additive genetic variation. This finding suggests that the greater morphological variability generally observed at the distal ends of tooth classes may be due primarily to a difference between the environmental conditions experienced by earlier and later developing teeth within a morphological class. The cusp of Carabelli and groove pattern of the lower molars, on the first tooth of their respective classes, were uncorrelated and showed higher degrees of resemblance between relatives than the other three characters studied. These two characters would therefore seem to serve as relatively good and independent population discriminators.

INTRODUCTION

IT HAS long been recognized that teeth at the distal ends of morphological classes tend to be more variable than their anterior neighbours (DAHLBERG, 1945). As far as tooth size is concerned, there is evidence to suggest that this greater variability is due to a larger environmental component of variation rather than a larger genetic component. The evidence comes from the fact that estimates of total genetic variance and heritability of the width of lower molars in the mouse have been found to tend towards progressive reduction from first to third molars (BADER and LEHMANN, 1965; BADER, 1965), and, in man, estimates of heritability for the size of upper incisors, upper and lower premolars, and upper and lower molars, have tended to be lower in the more distal teeth within each class (LUNDSTRÖM, 1948; HUNTER, 1959). Also in man, the more mesial teeth of each class appear to be more highly correlated among themselves and with the rest of the dentition than the more distal teeth, suggesting a decrease of intrinsic control over tooth size from mesial to distal in each class (GARN, LEWIS and KEREWSKY, 1965).

* Present address : University of Cambridge, Department of Genetics, Milton Road, Cambridge CB4 1XH, England.

The apparently greater non-genetic variability of size shown by the more distal teeth in each class seems to be related to developmental sequence and to local interactions between teeth developing in a confined space. If in a given morphological class the teeth which develop early are large, then those which develop late tend to be small or absent, and vice versa (GRÜNEBERG, 1951; GREWAL, 1962; VAN VALEN, 1962; SOFAER, 1969; SOFAER *et al.*, 1971). The environmental component of variation therefore includes not only a contribution from the environment of the animal as a whole but also a possibly more important contribution from the local environment around the developing tooth. Restriction of the local environment, interactions between developing teeth and developmental sequence provide an explanation in man for the paradoxical association between greater non-genetic variation in the more distal teeth of each class and the more rapid evolutionary change in size shown by these teeth than by their anterior neighbours (SOFAER, BAILIT and MACLEAN, 1971).

Tooth variability is not only expressed in terms of size but also in terms of shape. Investigations into the basis of morphological variation could thus provide additional information about differences in the sources of variability between the more mesial and more distal members of each tooth class. Furthermore, such investigations could indicate the relative value of different morphological characters and of different teeth as population discriminators, a result which could serve to clarify the significance of previous ethnic comparisons and perhaps point the way to more efficient use of morphological variables in the future. It was with these points in mind that the present analysis was undertaken.

MATERIALS AND METHODS

The data were derived from an examination of dental casts of two Melanesian tribes: Nasioi, collected on the island of Bougainville in the Territory of Papua and New Guinea; and Kwoio, collected on Malaita, one of the British Solomon Islands. Each individual had at least one first-degree relative in the sample, the total of 229 individuals yielding 117 parent-offspring pairs and 146 sibling pairs for both tribes combined.

The variables being considered are listed in Table 1. Only two categories of expression were used, so that for each character each tooth on each side was either non-affected and scored as 0, or affected and scored as 1. For each character for each tooth, each individual was given a score equal to the mean of the scores of the two sides, or, in cases where scoring was possible on one side only, to the score on this side.

TABLE 1. THE MORPHOLOGICAL CHARACTERS, THE TWO CATEGORIES IN WHICH EACH WAS SCORED, AND THE FREQUENCIES OF THE AFFECTED CONDITIONS

Character	Tooth	Non-affected	Affected	Number of teeth scored on both sides	Frequency of affected teeth
Shovel form	UI1 (Upper central incisor)	No trace of rim or fossa	All degrees of shovel form	358	0·84
	UI2 (Upper lateral incisor)			357	0·78
Cusp of Carabelli	UM1 (Upper first molar)	No feature	All degrees from pit, groove or ridge to pronounced cusp	394	0·27
	UM2 (Upper second molar)			342	0·12
Cusp number	UM2 (Upper second molar)	3 cusps	4 cusps or more	350	0·62
	UM3 (Upper third molar)			167	0·28
Cusp number	LM1 (Lower first molar)	4 cusps	5 cusps or more	391	0·79
	LM2 (Lower second molar)			343	0·28
Groove pattern	LM1 (Lower first molar)	+ pattern	Y or X pattern	350	0·67
	LM2 (Lower second molar)			305	0·20

Two aspects of morphological variation were studied: the relationships among characters and among teeth, investigated by calculating correlations between characters and between teeth within individuals, and the resemblance between relatives, analysed by calculating the intra-class correlation for each character for each tooth among all parent-offspring pairs and all sibling pairs. For the analysis of resemblance between relatives, the basis of morphological variation was assumed to be multifactorial, as there is at present no evidence to the contrary (SOFAER, 1970).

RESULTS

The correlations between characters for each tooth, and correlations between teeth within characters, are shown in Table 2. Among the correlations between characters, there was an overwhelmingly large number of positive coefficients. Even though few of these were significant, it seems that there may have been some general factor, perhaps tooth size, causing this overall tendency. Correlations between the morphological characters and mesiodistal tooth diameter were in fact all found to be positive, 8 out of 10 being significant at the 5 per cent level or better. Returning to Table 2, the only consistently significant association among the morphological

TABLE 2. CORRELATIONS BETWEEN CHARACTERS FOR EACH TOOTH, AND CORRELATIONS BETWEEN TEETH WITHIN CHARACTERS

Character		Shovel form		Cusp of Carabelli		Cusp number		Cusp number		Groove pattern	
	Tooth	UI1	UI2	UM1	UM2	UM2	UM3	LM1	LM2	LM1	LM2
Shovel form	UI1										
	UI2	0·67*									
Cusp of Carabelli	UM1	0·11	0·20†								
	UM2	0·01	0·15	0·65*							
Cusp number	UM2	0·07	0·08	0·12	0·04						
	UM3	0·02	0·03	0·07	0·12	0·23†					
Cusp number	LM1	0·14	0·16†	0·11	0·08	0·13	0·13				
	LM2	0·12	0·18†	0·10	0·00	0·17†	0·08	0·35*			
Groove pattern	LM1	0·03	0·04	0·06	0·03	0·20†	0·07	0·31*	0·20†		
	LM2	0·12	0·12	0·11	0·05	0·14	0·35*	0·20†	0·24*	0·29*	

* $P \leq 0{\cdot}01$.
† $P \leq 0{\cdot}05$.

characters themselves was that between cusp number and groove pattern of the lower molars. Correlations within characters between teeth of the same class were all positive and significant, suggesting a degree of common basis for variation of each character within a tooth class. They were particularly high for shovel form of the incisors and for the cusp of Carabelli.

Correlations between pairs of relatives are shown in Table 3. The t values indicate the significance and direction of the difference between correlations for siblings and correlations for parents and their offspring. Two differences were of borderline significance. In both, siblings were more alike than parents and their offspring, suggesting a possible effect of dominance or common sibling environment (FALCONER, 1964). However, as any such effect must have been small and was not general for all characters, the correlations between siblings and those between parents and their offspring were pooled to give a single set of correlations for first-degree relatives. Insofar as members of each pair of such relatives have, on average, half their genetic material in common with each other, they can be considered to form a homogeneous group.

TABLE 3. CORRELATIONS BETWEEN RELATIVES SHOWING THE DIFFERENCE BETWEEN SIBLINGS AND PARENTS AND THEIR OFFSPRING

Character	Tooth	Sibs		Parents and offspring		
		N	*r*	*N*	*r*	*t*
Shovel form	UI1	100	0·17	57	0·20	−0·23
	UI2	99	0·02	55	0·12	−0·57
Cusp of Carabelli	UM1	120	0·41	87	0·28	0·99
	UM2	93	0·29	51	−0·06	1·97*
Cusp number	UM2	99	0·02	54	0·29	−1·59
	UM3	44	0·10	11	−0·18	0·71
Cusp number	LM1	123	0·18	70	0·18	0·01
	LM2	101	0·25	45	−0·02	1·53
Groove pattern	LM1	97	0·31	60	0·34	−0·22
	LM2	78	0·35	36	−0·05	1·99*

N = number of pairs; *r* = correlation coefficient; *t* indicates the significance and direction of the difference between *r* for sibs and *r* for parents and their offspring.
* $P \approx 0{\cdot}05$.

The correlations between first-degree relatives, for each character, for early and late developing teeth within each class, are shown in Table 4. If the relationships among these correlations are an indication of common relationships for all populations, it follows that the cusp of Carabelli and groove pattern of the lower molars, on the first tooth of their respective classes, would serve as relatively good and independent population discriminators, since they show the greatest degree of resemblance between relatives (Table 4) and appear to be uncorrelated (Table 2). The high correlation between relatives shown by the cusp of Carabelli is consistent with previous recognition of this character as a good ethnic marker (PINTO-CISTERNAS and FIGUEROA, 1968).

TABLE 4. CORRELATIONS BETWEEN FIRST-DEGREE RELATIVES SHOWING THE DIFFERENCE BETWEEN EARLY AND LATE DEVELOPING TEETH FOR EACH CHARACTER

Character	Tooth pair	Early	Late	*t*
Shovel form	UI1 and UI2	0·18	0·06	1·10
Cusp of Carabelli	UM1 and UM2	0·35	0·17	1·81
Cusp number	UM2 and UM3	0·12	0·05	0·42
Cusp number	LM1 and LM2	0·18	0·17	0·04
Groove pattern	LM1 and LM2	0·32	0·24	0·75

t indicates the significance and direction of the difference between early and late.

The *t* values in Table 4 summarize the significance and direction of the difference between early and late teeth for each character. Although none of the five differences was itself significant, all were in the same direction, the correlation shown by the later developing tooth of each class being lower than that shown by its earlier developing partner. Thus it appears likely that, for the characters and teeth studied here, there is a real general tendency for the proportion of variation due to additive genetic effects to be lower in the later developing tooth than in the earlier developing tooth of the same class. If therefore seems reasonable to suppose that the environmental component of variation tends to be greater in the later developing tooth of a given class.

CONCLUSIONS

The five morphological characters studied here showed low, generally non-significant, but consistently positive within individual correlations one with another; possibly due to a general effect of tooth size. Correlation between characters was consistently significant only for the relationship of cusp number of the lower molars to groove pattern of the lower molars, suggesting a more intimate developmental relationship between these characters than between any others. Correlation within characters between teeth of the same class was significant for all characters, suggesting a degree of common basis for variation of each character within a tooth class. For all characters, the consistently lower correlation between relatives for the later developing more distal tooth in each class indicated that the greater morphological variation generally observed at the distal ends of tooth classes is probably due to a larger environmental component rather than a larger genetic component, the environmental component receiving contributions from the local environment of the developing tooth as well as from the environment of the animal as a whole. The cusp of Carabelli and groove pattern of the lower molars, on the first tooth of their respective classes, were uncorrelated and showed higher degrees of resemblance between relatives than the other three characters studied. These two characters would therefore seem to serve as relatively good and independent population discriminators.

Résumé—L'analyse de la ressemblance entre des parents de deux populations mélanésiennes indique que, pour chacun des cinq caractères morphologiques étudiés sur deux dents d'un même groupe, se formant successivement, la dent formée en dernier présente moins de variation génétique additive. Il semble que la variabilité morphologique généralement observée, à l'extrémité distale des groupes dentaires, peut être due principalement à la différence entre les conditions d'environnement s'exerçant sur les dents. Le tubercule de Carabelli et le type de sillons des molaires inférieures, de la première dent des classes respectives, ne présentent pas de corrélation et présentent un plus haut degré de ressemblance entre parents que les trois autres caractères étudiés. Ces deux caractères pourraient donc servir à discriminer deux populations indépendantes.

Zusammenfassung—Die Ähnlichkeitsanalyse zwischen Verwandten aus zwei melanesischen Bevölkerungsgruppen ergab, daß bei jeder von 5 morphologischen Eigenheiten eines früher und eines später sich entwickelnden Zahnes der später entwickelnde einen kleineren Anteil zusätzlicher genetischer Variationen aufwies. Dieser Befund deutet daraufhin, daß die allgemein an den distalen Enden der Zahnklassen beobachtete größere Variabilität in erster Linie auf unterschiedliche Milieubedingungen zurückzuführen sind, die auf die sich früher und später entwickelnden Zähne innerhalb einer morphologischen Klasse einwirken. Der Carabelli-Höcker und das Fissurenbild der unteren Molaren am ersten Zahn ihrer entsprechenden Klassen waren nicht miteinander korreliert und zeigten zwischen Verwandten höhere Ahnlichkeitsgrade als die anderen drei untersuchten Eigenheiten. Die beiden genannten Eigenschaften scheinen deshalb ein relativ gutes und unabhängiges Unterscheidungsmerkmal zu sein.

REFERENCES

BADER, R. S. 1965. Heritability of dental characters in the house mouse. *Evolution* **19**, 378–384.

BADER, R. S. and LEHMANN, W. H. 1965. Phenotypic and genotypic variation in odontometric traits of the house mouse. *Am. Midl. Nat.* **74**, 28–38.

DAHLBERG, A. A. 1945. The changing dentition of man. *J. Am. dent. Ass.* **32**, 676–690.

FALCONER, D. S. 1964. *Introduction to Quantitative Genetics*, Chap. 9. Oliver & Boyd, Edinburgh.

GARN, S. M., LEWIS, A. B. and KEREWSKY, R. S. 1965. Size interrelationships of the mesial and distal teeth. *J. dent. Res.* **44**, 350–354.

GREWAL, M. S. 1962. The development of an inherited tooth defect in the mouse. *J. Embryol. exp. Morph.* **10**, 202–211.

GRÜNEBERG, H. 1951. The genetics of a tooth defect in the mouse. *Proc. R. Soc.* B **138**, 437–451.

HUNTER, W. S. 1959. The inheritance of mesiodistal tooth diameter in twins, Ph.D. Thesis, University of Michigan, Ann Arbor.

LUNDSTRÖM, A. 1948. *Tooth Size and Occlusion in Twins.* S. Karger, New York.

PINTO-CISTERNAS, J. and FIGUEROA, H. 1968. Genetic structure of a population of Valparaiso. II. Distribution of two dental traits with anthropological importance. *Am. J. Phys. Anthrop.* **29**, 339–348.

SOFAER, J. A. 1969. Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *J. Embryol. exp. Morph.* **22**, 181–205; II. Observations on the reaction to changes of genetic background. *J. Embryol. exp. Morph.* **22**, 207–227.

SOFAER, J. A. 1970. Dental morphologic variation and the Hardy–Weinberg Law. *J. dent. Res.* **49**, 1505–1508.

SOFAER, J. A., BAILIT, H. L. and MACLEAN, C. J. 1971. A developmental basis for differential tooth reduction during hominid evolution. *Evolution* **25**, 509–517.

SOFAER, J. A., CHUNG, C. S., NISWANDER, J. D. and RUNCK, D. W. 1971. Developmental interaction, size and agenesis among permanent maxillary incisors. *Hum. Biol.* **43**, 36–45.

VAN VALEN, L. 1962. Growth fields in the dentition of Peromyscus. *Evolution* **16**, 272–278.

Reprinted from AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY
Vol. 37, No. 3, November 1972

Population Studies on Southwestern Indian Tribes

V. TOOTH MORPHOLOGY AS AN INDICATOR OF BIOLOGICAL DISTANCE

J. A. SOFAER,[1] J. D. NISWANDER, C. J. MacLEAN AND P. L. WORKMAN [2]
Human Genetics Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014

KEY WORDS Teeth · Morphology · Distance · American Indians.

ABSTRACT The value of phylogenetic comparisons between populations based on tooth morphology depends on a knowledge of the extent to which the observed morphological variation is genetic in origin. This knowledge can be derived unequivocally only from the analysis of family data. However, in the absence of such knowledge the ability of tooth morphology to distinguish biological differences can be evaluated directly by testing its discriminating power in practice on populations between which the degrees of genetic difference are already known. The results of such an evaluation show that different degrees of subjectivity of scoring are associated with different characters, but that moderately good correspondence between known genetic differences and differences based on tooth morphology can be achieved when characters showing the least subjectivity of scoring are used.

The unique qualities that make teeth valuable for evolutionary studies are well known. Teeth are among the most durable parts of the body and accordingly constitute a large proportion of the human and prehuman fossil remains available for study. In living populations they are readily accessible and, if required, permanent and accurate records in the form of casts can be made with little difficulty. Dental characteristics can therefore be used to compare both past and present populations. However, a difficulty involved in tooth based comparisons that has not been sufficiently stressed in the past is that the significance of such comparisons is not clear unless the underlying causes of the observed variation are understood. A primary requirement in drawing valid conclusions is a knowledge of the extent to which the observed variation is genetic in origin.

A small amount of experimental evidence from the mouse indicates that dental morphological variation has a considerable genetic component. Morphological variants are characteristic of different inbred strains maintained under the same environmental conditions (Grüneberg, '65; Sofaer, '69a); and specific dental morphological anomalies have been shown to result from known allele substitutions at single loci, the effects of these allele substitutions being subject to modification by genetic background (Grüneberg, '65; Sofaer, '69b). In man also, at least some proportion of the observed variation appears to be genetic. Human morphological anomalies of the teeth have been associated with syndromes of hereditary origin (Gorlin and Pindborg, '64); and limited twin studies have demonstrated a greater concordance of dental morphological characters within monozygotic pairs than within dizygotic pairs (Korkhaus, '30; Ludwig, '57; Saheki, '58; Lundström, '63). The actual mode of genetic control of these characters has yet to be established, though there have been attempts to support the suggestion that particular human dental morphological variants are controlled by single autosomal loci. These attempts have involved the use of a few small pedigrees (Kraus, '51; Tsuji, '58), or the application of the Hardy-Weinberg Law to population frequencies of arbitrar-

[1] Present address: University of Cambridge, Department of Genetics, Milton Road, Cambridge, England.
[2] Present address: Department of Anthropology, University of Massachusetts, Amherst, Massachusetts 01002.

ily defined levels of expression of each character (Turner, '67; '69; Devoto et al., '68). However, no firm conclusion can be drawn from these pedigrees, and the population approach alone is certainly not a valid one (Sofaer, '70). Clearly, a present need is to accumulate good family data. If no simple genetic model is then found to be applicable, quantitative methods can be applied to establish the degree of reliance that can be placed on a particular variant or combination of variants as an indicator of a genetic difference.

In the absence of good family data population comparisons based on tooth morphology must be interpreted with caution. However, it may be possible to evaluate directly the ability of tooth morphology to distinguish biological differences by testing its discriminating power in practice on populations between which the degrees of genetic difference have already been estimated by the study of gene frequencies for a number of simple genetic polymorphisms. The present paper is concerned with such an approach.

The population comparisons described here are of two different kinds. Firstly, a comparison is made between the Pima, Papago and Zuni, three North American Indian tribes of the Southwestern United States. The Pima and Papago are closely related desert agriculturalists with similar languages and cultures who occupy neighboring reservations in Arizona. The Zuni, who live on a reservation in Northwestern New Mexico, are a Western Pueblo people, relatively distantly related to the Pima and Papago (Niswander et al., '70; Workman et al., unpublished manuscript). To evaluate the usefulness of tooth morphology as an indicator of microevolutionary change, tooth based distances between these tribes are compared with geographic distances and with estimates of genetic difference calculated by Workman et al. (unpublished manuscript) from blood group and serum protein variant frequencies (Brown and Johnson, '70). Secondly, tooth based distances between seven broad world population groups are calculated from data taken from the literature. These distances are then considered in the light of current thoughts on the relationships between peoples, bearing in mind that the data have been drawn from a number of different sources.

MATERIAL AND METHODS

Original data were derived from intraoral examinations of 610 Zunis (380 females and 230 males), and from the examination of dental casts of 327 Pimas (156 females and 171 males), and 164 Papagos (98 females, 55 males and 11 of unknown sex). Scoring was restricted to two categories, "non-affected" and "affected," for each of ten morphological characters. For present purposes the ten characters are assumed to be independent — an assumption which is not strictly true but of little importance for consideration of relative distances. Teeth in which the characters could have been obliterated by wear or restoration were not included. All individuals were scored by the same observer.

Many dental morphological characters behave as quasi-continuous variables; that is, they are either present or absent, but when present they vary continuously from the lowest level of expression to the highest. The accepted model of quasi-continuous variation is based on the assumption that there is an underlying scale of continuous variation of some attribute (a combination of all the genetic and environmental factors involved) that is immediately related to the development of the character. Individuals in the non-affected class occupy positions on this scale below a threshold value, and affected individuals occupy positions above the threshold value. The higher the position above the threshold the more intense the expression of the character (Grüneberg, '52). All-or-none classification is thus a somewhat arbitrary division of a continuous scale. If such a model applies to the characters considered here, then different populations can be regarded as being distributed over different ranges of the underlying continuous scale. The most important characteristic of a population is the mean of its distribution on this scale. The relative positions of different population means are reflected in the population frequencies of the affected class, assuming a common variance for all populations. Further subclassification of affected individuals gives no additional information about the mean, though

it could be used to test for equality of variance between populations. However, subdivision of the affected class increases the opportunity for misclassification, and is likely to exaggerate any differences between scoring from intraoral examination and from casts. This latter source of possible error could only be evaluated by taking casts of a sample of individuals who were scored by intraoral examination, a procedure for which there were insufficient resources.

The scoring of morphological characters is known to be a subjective evaluation and therefore open to differences of interpretation, not only between observers, but also within a single observer from one scoring session to the next. In order to establish the relative degrees of subjectivity of scoring associated with the different characters the Papago casts were scored on two different occasions separated by several months. During this period the observer was not involved in any similar scoring procedures.

The measure of distance applied to both the dental and genetic data was Sanghvi's X^2. This is a chi squared statistic that provides a mean measure of divergence, enabling estimates of divergence based on different numbers of characters, each possibly scored in a different number of categories, to be compared directly (Sanghvi, '53). The measure is equal to:

$$X^2 = \frac{\sum_{1}^{n}\sum_{1}^{r}\left[\frac{(P_1-Q)^2}{Q}+\frac{(P_2-Q)^2}{Q}\right]}{\text{d.f.}}$$

where P_1 and P_2 are the percentage incidences in two populations of each of r classes in which a given character is scored, where $Q = (P_1 + P_2)/2$, where n is the number of characters scored, and where d.f. $= n(r-1)$. (Since in this study $r = 2$ in every case, d.f. $= n$).

The requirements of a true distance function, listed by Rao ('52), include that the distance between any two of three populations should not be greater than the sum of the two distances between each of these populations and the third. This is known as the triangle law of distance, and it must apply if the relationships between populations are to be plotted graphically in a conventional way. Sanghvi's X^2, although a valid measure of divergence, is not a true distance function since it violates the triangle law. However, the square root of this measure does not violate the triangle law and was therefore chosen as the basis of the present comparisons.

RESULTS

The characters, the two categories in which each was scored, the number of individuals in whom scoring was possible, and the percentages of affected teeth (both sides combined) are shown for the three Indian tribes in table 1. Table 2 shows the result of scoring the Papago casts on two separate occasions, and lists the characters in order of decreasing concordance (frequency of identical scores on both occasions). The low concordance shown by the cusp of Carabelli was a surprising finding since previous studies have suggested that this character is a good ethnic marker (Pinto-Cisternas and Figueroa, '68) and that it shows a relatively high component of additive genetic variation in another population (Sofaer, Bailit and MacLean, '72).

The considerable lack of repeatability associated with some of the characters suggested that small differences between tribes might be masked by inconsistent scoring. Accordingly, Sanghvi's X^2 was calculated for different numbers of characters, progressively reducing the number of characters by removing those showing the poorest repeatability. The results of these calculations are shown in table 3. Also given in this table are total χ^2 values, which test the significance of the differences between tribes. They are the sum of χ^2 values for the test of homogeneity (each with one degree of freedom) calculated for the characters individually.

Triangles of relative distance based on different numbers of dental morphological characters are shown in figure 1 together with triangles showing the relative geographic distances between tribes and the relative estimates of genetic distance, based on gene frequencies at 12 loci (Workman et al., unpublished manuscript). The numbering of the dental characters is according to table 2.

TABLE 1

The characters, the two categories in which each was scored, the numbers of individuals scored (N), and percentages of affected teeth (% A) in the three tribes [1]

Tooth	Character	Non-affected	Affected	Number of individuals scored and per cent affected teeth					
				Zuni		Pima		Papago	
				N	% A	N	% A	N	% A
Upper central incisor (UI1)	Palatal shovelling	No trace of rim or fossa	All degrees of shovelling	549	94.4	325	97.5	148	97.3
	Labial shovelling	No trace of labial ridging on either side	All degrees of ridging	549	13.6	324	11.6	147	30.9
Upper lateral incisor (UI2)	Barrel shape	Normal spatulate incisor	Barrel shaped	548	3.0	320	6.7	149	6.5
Upper first molar (UM1)	Cusp of Carabelli	No feature	All degrees from pit, groove or ridge to pronounced cusp	517	36.2	322	53.3	146	44.6
Upper second molar (UM2)	Cusp number	3 cusps	4 cusps (including 3+)	531	66.7	237	81.8	138	75.6
Lower first molar (LM1)	Protostylid	No feature	All modifications of buccal surface of mesiobuccal cusp	520	0.1	323	7.8	140	3.3
	Groove pattern	+ pattern	Y or X pattern	187	84.5	265	94.8	108	78.0
	Cusp number	5 cusps or less	6 cusps or more	516	4.0	322	17.4	142	5.1
Lower second molar (LM2)	Groove pattern	+ pattern	Y or X pattern	274	5.6	230	10.6	132	5.2
	Cusp number	4 cusps	5 cusps or more	523	34.9	258	45.5	132	24.3

[1] Scoring was based on the descriptions of Dahlberg, ('49, '63) with the "non-affected" and "affected" classes as described above.

The percentage incidences of the affected condition for five of the characters already described are shown for seven world populations in table 4. The references from which these data were drawn are listed under DATA REFERENCES at the end of the paper. In some cases the only sample size information given was the number of teeth on which the frequency estimate was based. In these cases it was assumed that both right and left teeth were scored for each individual and accordingly N was taken as half the number of teeth. The effective sample size was taken as the number of individuals rather than the number of teeth since the two sides of the body of an individual are phenotypic expressions of a single genotype. The "Caucasian" population contains European and North American white groups, the "Negro" population contains African and North American black groups, and the "Semitic" population contains Bedouin, and Jews from Yemen and Cochin. For each population table 4 gives low and high extreme frequency estimates, and a weighted mean estimate of the frequency of the affected condition for each character. The combined Zuni-Pima-Papago frequencies are shown for comparison.

Distances between all pairs of populations listed in table 4 are shown in fig-

TABLE 2

Concordance of scoring 164 Papago casts on two occasions. Characters ranked according to degree of concordance

Character	Concordance
1. UI2 Barrel shape	0.99
2. UI1 Palatal shovelling	0.99
3. UM2 Cusp number	0.94
4. LM1 Protostylid	0.94
5. LM2 Groove pattern	0.93
6. LM1 Cusp number	0.93
7. UI1 Labial shovelling	0.88
8. LM2 Cusp number	0.85
9. LM1 Groove pattern	0.79
10. UM1 Cusp of Carabelli	0.78

TABLE 3

Measures of distance based on different numbers of characters

Characters (numbered as in table 2)	Tribal pair	Significance tests χ^2 (d.f. = N [3])	Sanghvi X^2	Distance [1]
	Zuni-Pima	153	4.17	1.38
1–10	Zuni-Papago	52	2.18	1.00
	Pima-Papago	84	4.72	1.47
	Zuni-Pima	118	3.76	1.26
1–8	Zuni-Papago	47	2.37	1.00
	Pima-Papago	59	4.21	1.33
	Zuni-Pima	110	4.60	1.91
1–6	Zuni-Papago	19	1.26	1.00
	Pima-Papago	18	2.10	1.29
	Zuni-Pima	65	4.16	2.31
1–4	Zuni-Papago	19	1.85	1.54
	Pima-Papago	4	0.78	1.00
	Zuni-Pima	10	1.40	11.83
1–2	Zuni-Papago	5	1.20	10.95
	Pima-Papago	0	0.01	1.00
Genetic distance [2] from blood typing		d.f.		
	Zuni-Pima	15	1.94	1.52
	Zuni-Papago	17	2.42	1.70
	Pima-Papago	15	0.84	1.00

[1] $\sqrt{X^2}$ scaled by setting smallest calculated figure in each series equal to 1.
[2] Data from Workman et al., based on ABO, MNSs, Rh (4 classes) P, Fy, JK, Di, Hp, Tf, AL. Gc and cerumen used in Zuni-Papago calculation only.
[3] N, number of characters.

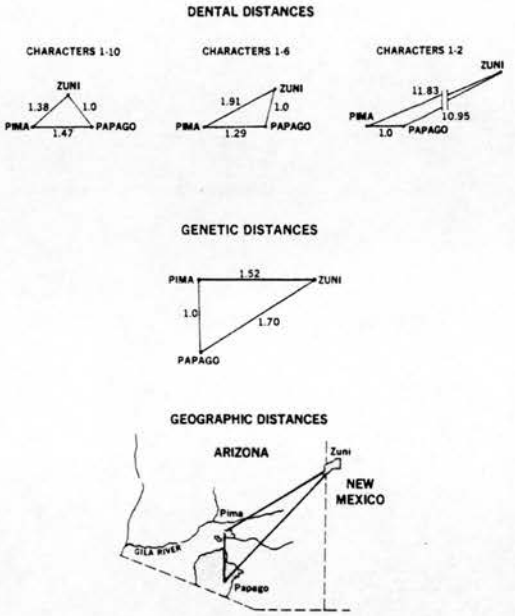


Fig. 1 Dental, genetic, and geographic distance among three southwestern Indian tribes. Dental distances are calculated for different numbers of characters.

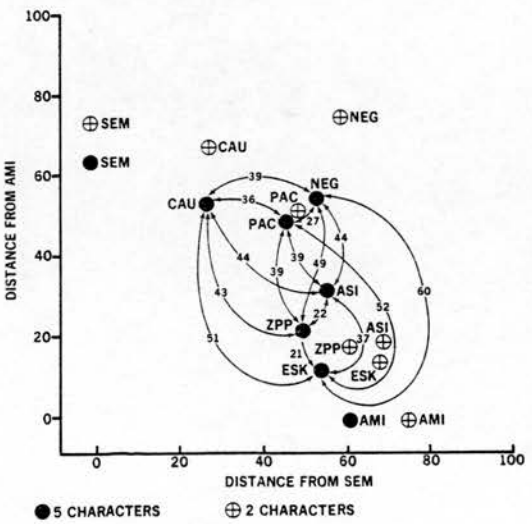


Fig. 2 Dental distances among several world populations based on data in table 4. Scale is ten times the calculated values.

ure 2. The distances are based on the weighted mean frequency estimates. The two axes of the plot in figure 2 were chosen because the distance between the American Indian and Semitic populations was greater than any other. One set of points in figure 2 shows the relative positions of the populations based on all five characters, and the other set shows the positions based on only the two characters judged by the present investigation to be scored with the greatest reliability (UI1 palatal shovelling and UM2 cusp number — see table 2). The general relationship among the populations is similar for both sets of points. There is no overlap between the mongoloid populations (American Indian; Zuni, Pima and Papago; Eskimo; Asian) which occupy the lower right portion of the plot, and the non-mongoloids (Semitic; Caucasian; Pacific and Australian; Negro) which occupy the upper left portion of the plot. Furthermore, within the mongoloids the relationship of Asians and Eskimos to the American Indians is consistent with current thoughts on the origin of the American Indians. The fact that the Zuni, Pima and Papago are out of line could be attributed to observer bias, values for the other three mongoloid populations being based on means of data from a number of sources. Among the non-mongoloids the Caucasians are closest to the Semitic population, and the Pacific and Australian group is closest to the Negroes, both of which are not unexpected relationships.

The effect of removing those characters showing poor repeatability of scoring is to increase the separation between the mongoloid and non-mongoloid groups, and to close the distances within the mongoloids. It is possible that this change is an indication that the two chosen characters are providing better discrimination than all five taken together.

Having considered each type of population comparison alone it is relevant to compare the inter-tribal distances with those separating the different world populations. The two world populations most closely related to the Zuni, Pima and Papago are the American Indians (composed of samples from different areas of North and South America) and the Eskimos. The $\sqrt{X^2}$ distance between these two populations, based on weighted mean frequency estimates for the two best dental characters, is 15.1, the smallest mean distance for all pairs of populations in figure 2 except that separating the Zuni, Pima and Papago combined from the Eskimos. This can be compared with a Zuni-Pima dis-

TABLE 4

Low, high and weighted mean estimates of the percentage incidence of the affected condition, % A, for five morphological characters in different world populations. N is the number of individuals on which each estimate was based. References are listed under DATA REFERENCES *at the end of the paper*

Character	Population code:	ZPP Zuni-Pima -Papago		AMI American Indian			ESK Aleut and Eskimo			ASI Asia		
	Estimate of % A	N	% A	N	% A	References	N	% A	References	N	% A	References
UI1 Palatal shovelling	Low			342	100.0	6, 12	499	99.2	20	269	85.0	17
	High			342	100.0	6, 12	267	100.0	3, 19, 21	259	97.7	27
	Mean	1022	95.8	342	100.0	6, 12	766	99.5	3, 19–21	1817	92.8	11, 17, 27
UM1 Cusp of Carabelli	Low			41	12.0	6	60	13.3	19	339	31.9	28
	High			200	83.5	7	61	78.3	29	339	31.9	28
	Mean	985	43.1	844	60.2	6, 7, 9, 16, 30	384	66.3	19, 29, 30	339	31.9	28
UM2 Cusp number	Low			53	66.2	9	91	65.7	21	887	84.6	26
	High			97	91.8	30	118	72.9	30	887	84.6	26
	Mean	906	72.0	241	82.1	6, 9, 30	264	69.6	19, 21, 30	887	84.6	26
LM1 Groove pattern	Low			53	76.9	9	29	41.4	19	40	100.0	10
	High			55	100.0	10	30	97.0	10	40	100.0	10
	Mean	560	88.3	270	95.1	6, 9, 10	202	88.2	10, 19, 21	40	100.0	10
LM2 Cusp number	Low			55	32.0	10	30	43.0	10	19	19.0	10
	High			53	72.0	9	58	66.1	21	21	31.0	10
	Mean	913	36.3	197	60.4	6, 9, 10	124	57.4	10, 19, 21	40	25.3	10

Character	Population code:	PAC Pacific and Australia			CAU Caucasian			NEG Negro			SEM Semitic		
	Estimate of % A	N	% A	References	N	% A	References	N	% A	References	N	% A	References
UI1 Palatal shovelling	Low	167	41.0	22	100	17.0	3	264	16.6	25	137	41.5	24
	High	59	88.1	11	212	91.0	15	807	44.4	11	60	47.0	23
	Mean	1045	56.8	1, 11, 22, 27	1833	40.5	3, 11, 15, 17, 27	1193	37.2	3, 11, 25	197	43.2	23, 24
UM1 Cusp of Carabelli	Low	67	19.4	1	91	41.0	6	389	2.0	25	30	62.0	23
	High	30	33.0	2	140	85.7	7	274	57.7	16	30	93.0	23
	Mean	97	23.6	1, 2	3789	59.5	6–8, 14, 16, 18	663	25.0	16, 25	197	73.9	23, 24
UM2 Cusp number	Low	53	69.8	1	53	58.0	6	78	100.0	25	137	30.5	24
	High	104	100.0	2	50	87.5	7	78	100.0	25	30	73.0	23
	Mean	265	88.7	1, 2, 5	103	72.3	6, 7	78	100.0	25	197	42.1	23, 24
LM1 Groove pattern	Low	57	54.9	1	85	86.0	10	133	86.9	4	30	53.0	23
	High	20	100.0	10	75	96.0	6	49	100.0	10	137	70.4	24
	Mean	77	66.6	1, 10	221	91.6	6, 10	182	90.4	4, 10	197	65.7	23, 24
LM2 Cusp number	Low	97	12.5	5	61	1.0	10	167	18.6	4	60	0	23
	High	20	48.0	10	356	14.0	13	69	53.7	25	137	7.0	24
	Mean	232	24.6	1, 2, 5, 10	611	11.0	10, 13	285	28.2	4, 10, 25	197	4.9	23, 24

tance of 11.83, a Zuni-Papago distance of 10.95, and a Pima-Papago distance of 1.00.

DISCUSSION

The subjectivity associated with scoring morphological characters, which has been recognized as a problem in the past, is emphasized here by the results of both kinds of population comparison. The results from the Indian data show that, for two of the ten characters, even when scoring each character in two categories only in the hope of reducing subjective error, the same observer without permanent standards of reference could only score the same individual twice in the same way about 80% of the time. It is perhaps worth restating at this point that scoring each character in more than two classes could only increase the frequency of error. It is also true that more experienced observers would be likely to show a higher repeatability, and conversely those with less experience might do more poorly. This demonstrates the necessity for standardized scoring conditions, and the value of standardized reference plaques showing the bounds of each category being scored. On the other hand, certain of the characters showed high repeatability of scoring in spite of the possible shortcomings of the scoring procedure. These characters presumably would also be scored more consistently by different observers, and are therefore likely to be more reliable variables when using data from different sources.

Even though the low repeatability of some of the characters was a disquieting finding, the consistent change in the tooth based triangles in figure 1 with progressive removal of the characters showing the poorest repeatability was encouraging. This change, towards a relative closeness between the Pima and Papago, and their mutual distance from the Zuni, the fundamental relationship shown by geography and by the genetic estimates, suggests that tooth morphology has the potential of providing moderately good discrimination, even on the fine level on which the three Indian tribes are related. It should be pointed out, however, that four of the ten characters listed in table 1 show relative frequencies in the three tribes contrary to the expectation based on geographic relationships and the study of gene frequencies; that is, the Zuni are intermediate between the Pima and Papago. In the two instances of labial shovelling and LM2 groove pattern the results are so clearly at odds with the expectation that some doubt could be cast on the importance of genetic factors in the variability of these traits. All four characters are among those with the lowest repeatability and, until more information is available on their genetic basis, they should perhaps be considered among the least useful for population discrimination. The near equilateral quality of the triangles based on all characters is therefore consistent with the assumption that, in these triangles, distances were composed largely of equal amounts of random error which masked the true relationship between tribes. Only after the majority of this error was removed did a relationship approaching the geographic and genetic relationships begin to emerge.

Various combinations of the low and high extreme frequency estimates in table 4 were used to calculate the minimum and maximum possible distances between each pair of populations that would be obtained if individual frequency estimates were used. Minimum and maximum distances ranged from one-fourth to over four times the distance based on the weighted mean frequencies. These widely disparate and often inconsistent results contrast with the relatively reasonable picture that emerges when the mean frequencies are used. This emphasises the need for caution when using single estimates from different sources to draw inferences about population relationships.

In conclusion then, it seems that the value of tooth morphology as an indicator of genetic differences between populations can be viewed with cautious optimism. However, careful standardization of the scoring procedure is clearly an important prerequisite to the collection of meaningful data. Analysis of family data is still urgently required, and should allow the selection of characters showing the greatest genetic variation. Such characters can be expected to be the best discriminators of genetic differences between populations.

The comparison between world populations made here has shown that there is a great deal of variation between different estimates of the frequency of a particular variant in a particular population; and that when population comparisons are made using data from different sources, the mean of several estimates is likely to be considerably more valuable than any one estimate alone.

ACKNOWLEDGMENTS

The authors would like to thank Dr. A. A. Dahlberg for access to his collection of Pima casts, and the Zuni Indians for their patient and cheerful cooperation. The Papago material was collected during the course of recent studies made by the Human Genetics Branch, National Institute of Dental Research.

LITERATURE CITED

Brown, K. S., and R. S. Johnson 1970 Population Studies on Southwestern Indian tribes. III. Serum protein variations of Zuni and Papago Indians. Hum. Hered., *20:* 281–286.

Dahlberg, A. A. 1949 The dentition of the American Indian. In: The Physical Anthropology of the American Indian. W. S. Laughlin, ed. Viking Fund., N.Y.

——— 1963 Analysis of the American Indian dentition. In: Dental Anthropology. D. Brothwell, ed. Pergamon Press, Oxford.

Devoto, F. C. H., N. H. Arias, S. Ringuelet and N. H. Palma 1968 Shovel-shaped incisors in a Northwestern Argentine population. J. Dent. Res., *47:* 820–823.

Gorlin, R. J., and J. J. Pindborg 1964 Syndromes of the Head and Neck. McGraw-Hill, N.Y.

Grüneberg, H. 1952 Genetical studies on the skeleton of the mouse. IV. Quasi-continuous variations. J. Genet., *51:* 95–114.

——— 1965 Genes and genotypes affecting the teeth of the mouse. J. Embryol. exp. Morph., *14:* 137–159.

Korkhaus, G. 1930 Anthropologic and odontologic studies of twins. Int. J. Orthodont., *16:* 640–647.

Kraus, B. S. 1951 Carabelli's anomaly of the maxillary molar teeth. Am. J. Hum. Genet., *3:* 348–355.

Ludwig, F. J. 1957 The mandibular second premolars: morphologic variation and inheritance. J. Dent. Res., *36:* 263–273.

Lundström, A. 1963 Tooth morphology as a basis for distinguishing monozygotic and dizygotic twins. Am. J. Hum. Genet., *15:* 34–43.

Niswander, J. D., K. S. Brown, B. Y. Iba, W. C. Leyshon and P. L. Workman 1970 Population studies on Southwestern Indian tribes. I. History, culture, and genetics of the Papago. Am. J. Hum. Genet., *22:* 7–23.

Pinto-Cisternas, J., and H. Figueroa 1968 Genetic structure of a population of Valparaiso. II. Distribution of two dental traits with anthropological importance. Am. J. Phys. Anthrop., *29:* 339–348.

Rao, C. R. 1952 Advanced Statistical Methods in Biometric Research. Wiley, N.Y.

Saheki, M. 1958 Heredity of the tooth crown configuration studies in twins. Acta Anat. Nippon., *33:* 456–470.

Sanghvi, L. D. 1953 Comparison of genetical and morphological methods for a study of biological differences. Am. J. Phys. Anthrop., *11:* 385–404.

Sofaer, J. A. 1969a The genetics and expression of a dental morphological variant in the mouse. Archs. Oral Biol., *14:* 1213–1223.

——— 1969b Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. J. Embryol. exp. Morph., *22:* 207–227.

——— 1970 Dental morphologic variation and the Hardy-Weinberg Law. J. Dent. Res., *49:* 1505–1508.

Sofaer, J. A., H. L. Bailit and C. J. MacLean 1972 Heredity and morphological variation in early and late developing human teeth of the same morphological class. Archs. Oral Biol., *17:* 811–816.

Tsuji, T. 1958 Incidence and inheritance of the Carabelli's cusp in a Japanese population. Jap. J. Hum. Genet., *3:* 21–31.

Turner, C. G. II. 1967 Dental genetics and microevolution in prehistoric and living Koniag Eskimo. J. Dent. Res., *46:* 911–917.

——— 1969 Microevolutionary interpretations from the dentition. Am. J. Phys. Anthrop., *30:* 421–426.

Workman, P. L., J. D. Niswander and W. C. Leyshon Population studies on Southwestern Indian tribes. IV. The Zuni. (Unpublished manuscript.)

DATA REFERENCES

1. Bailit, H. L., S. J. DeWitt and R. A. Leigh 1968 The size and morphology of the Nasioi dentition. Am. J. Phys. Anthrop., *28:* 271–288.
2. Campbell, T. D. 1925 The Dentition and Palate of the Australian Aboriginal. Sheridan Foundation, University of Adelaide.
3. Carbonell, V. M. 1963 Variations in the frequency of shovel-shaped incisors in different populations. In: Dental Anthropology. D. R. Brothwell, ed. Pergamon Press, Oxford.
4. Chagula, W. K. 1960 The cusps on the mandibular molars of East Africans. Am. J. Phys. Anthrop., *18:* 83–90.
5. Dahlberg, A. A. 1945 The changing dentition of man. J. Am. Dent. Assoc., *32:* 676–690.
6. ——— 1949 The dentition of the American Indian. In: The Physical Anthropology of the American Indian. W. S. Laughlin, ed. Viking Fund, N.Y.
7. ——— 1963 Analysis of the American Indian dentition. In: Dental Anthropology. D. R. Brothwell, ed. Pergamon Press, Oxford.
8. Dietz, V. H. 1944 A common dental morphotropic factor, the Carabelli cusp. J. Am. Dent. Assoc., *31:* 784–789.

9. Goaz, P. W., and M. C. Miller 1966 A preliminary description of the dental morphology of the Peruvian Indian. J. Dent. Res., *45:* 106–119.
10. Hellman, M. 1928 Racial characters in human dentition. Proc. Am. Phil. Soc., *67:* 157–174.
11. Hrdlička, A. 1920 Shovel-shaped teeth. Am. J. Phys. Anthrop., *3:* 429–465.
12. ——— 1931 Anthropology of the Sioux. Am. J. Phys. Anthrop., *16:* 123–166.
13. Jørgensen, K. D. 1955 The Dryopithecus pattern in recent Danes and Dutchmen. J. Dent. Res., *34:* 195–208.
14. Kallay, J. 1966 Extra cusp formation in the human dentition. J. Dent. Res., *45:* 1381–1394.
15. Koski, K., and E. Hautala 1952 On the frequency of shovel-shaped incisors in the Finns. Am. J. Phys. Anthrop., *10:* 127–132.
16. Kraus, B. S. 1959 Occurrence of the Carabelli trait in southwest ethnic groups. Am. J. Phys. Anthrop., *17:* 117–123.
17. Lasker, G. W., and M. M. C. Lee 1957 Racial traits in the human teeth. J. Forens. Sci., *2:* 401–419.
18. Meredith, H. V., and E. H. Hixon 1954 Frequency, size and bilateralism of Carabelli's tubercle. J. Dent. Res., *33:* 435–440.
19. Moorrees, C. F. A. 1957 The Aleut Dentition. Harvard U. Press, Cambridge.
20. Oschinsky, L., and R. Smithurt 1960 On certain dental characters of the Eskimo of the Eastern Canadian Arctic. Anthropologica, *2:* 105–112.
21. Pedersen, P. O. 1949 The East Greenland Eskimo dentition. Meddelelser om Gronland, *142:* 1–256, Copenhagen.
22. Riesenfeld, A. 1956 Shovel-shaped incisors and a few other dental features among the native peoples of the Pacific. Am. J. Phys. Anthrop., *14:* 505–521.
23. Rosenzweig, K. A., and Y. Zilberman 1967 Dental morphology of the Jews from Yemen and Cochin. Am. J. Phys. Anthrop., *26:* 15–22.
24. ——— 1969 Dentition of Bedouin in Israel. II. Morphology. Am. J. Phys. Anthrop., *31:* 199–204.
25. Shaw, J. C. M. 1931 The Teeth, the Bony Palate, and the Mandible in the Bantu Races of South Africa. Bale and Danielsson, London.
26. Suzuki, M., and T. Sakai 1956 On the occlusal surface patterns of cusps of maxillary molars in recent Japanese. J. Anthrop. Soc. Nippon, *65:* 54–61.
27. ——— 1964 Shovel-shape incisors among the living Polynesians. Am. J. Phys. Anthrop., *22:* 65–72.
28. Tsuji, T. 1958 Incidence and inheritance of the Carabelli's cusp in a Japanese population. Jap. J. Hum. Genet., *3:* 21–31.
29. Turner, C. G. II. 1967 Dental genetics and microevolution in prehistoric and living Koniag Eskimo. J. Dent. Res., *46:* 911–917.
30. ——— 1969 Microevolutionary interpretations from the dentition. Am. J. Phys. Anthrop., *30:* 421–426.

SUSCEPTIBILITY TO DISEASE

Mice with 'sex-linked anaemia', an inherited iron deficiency due to defective iron absorption, have thinner lingual epithelium than normal, and observations suggest that they may be more susceptible to oral candidosis. Different strains of candida produce different levels of oral colonisation and infection suggesting that differences in susceptibility to candidosis may result from variation in the microorganism as well as the host. In man, the results of a family study of Paget's disease of bone are consistent with the hypothesis that the disease is caused by a common and widespread virus, with genetic variation for susceptibility and perhaps severity of the disease.

Archs oral Biol. Vol. 26, pp. 343 to 344, 1981
Printed in Great Britain

0003-9969/81/040343-02$02.00/0
Pergamon Press Ltd

SHORT COMMUNICATION

LINGUAL EPITHELIAL THICKNESS IN MICE WITH INHERITED IRON-DEFICIENCY ANAEMIA (*sla*)

B. STEELE, J. A. SOFAER and J. C. SOUTHAM

University of Edinburgh, Department of Oral Medicine and Oral Pathology, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR, Scotland, U.K.

Summary—The mouse-mutant sex-linked anaemia (*sla*) suffers from iron-deficiency anaemia due to a defect of iron absorption from the gut. The lingual epithelium of anaemic mice was significantly thinner and less well-differentiated into papillae and rete pegs than normal when taken from the anterior dorsum but not from the posterior dorsum. The mutant may be a useful experimental model in which to study more detailed effects of iron deficiency on the oral mucosa.

Atrophy of the lingual mucosa has long been recognized as a sign of iron-deficiency anaemia, although the mechanisms involved remain a matter for speculation. The present study was carried out to determine whether the mouse-mutant sex-linked anaemia (*sla*) might be a suitable animal model in which to study the effect of iron deficiency on lingual epithelium, the advantage of the mutant over previous model systems being its consistent level of anaemia without the need for artificial induction through dietary restriction or bleeding.

Mice with sex-linked anaemia have a defect of iron transport in which intestinal cells take up iron but fail to pass it on normally to the circulation (Pinkerton, 1968; Edwards and Bannerman, 1970; Manis, 1971; Sorbie, Hamilton and Valberg, 1974). The anaemia is hypochromic and microcytic. In animals aged up to 200 days, plasma iron is reduced to approx. 1/3 of normal (Edwards *et al.*, 1977) the red-cell count approx. 80 per cent, packed cell volume 60–80 per cent and haemoglobin concentration 40–80 per cent of control values, the anaemia being most severe in younger animals and tending to lessen with age (Bannerman and Pinkerton, 1967). Iron stores, which can be demonstrated clearly in the spleens of normal mice by Prussian-blue staining, are almost invariably absent in mice with sex-linked anaemia, although occasionally detectable in trace amounts (Pinkerton, 1968). The basic biochemical defect is not known, but it is unlikely to be restricted to the intestinal mucosa because there is evidence for defective placental transport of iron also (Kingston, Bannerman and Bannerman, 1978).

Heterozygous female mice (*sla*/+) were mated to normal males (+/*Y*), the offspring expected being half heterozygous and half normal females (all normal with respect to the anaemia), and half anaemic (*sla*/*Y*) and half normal males. Male offspring only were studied. At ages from 57–99 days, blood was taken for investigation and the tongues and spleens removed and fixed in 10 per cent buffered formol saline. After embedding in paraffin wax, 5 μm thick sections of tongue and spleen were cut. For the tongue, these were taken sagittally in the region of the midline for some animals, or transversely at the junction of the anterior 2/3 and posterior 1/3 of tongue for others. Tongue sections were stained with haematoxylin and eosin, and spleen sections with Prussian blue. Mice were classified as normal or anaemic on the basis of the haematological findings and the presence or absence of a significant Prussian-blue reaction in sections of spleen.

For each mouse, measurements of dorsal tongue epithelium using a Kontron MOP AMO1 Image Analyser were made either on 3 consecutive serial sagittal sections, or on 2 consecutive serial transverse sections. For sagittal sections, a standard length, *L*, of epithelium was measured from an arbitrary point just posterior to the tip, backwards along the dorsal surface. The area, *A*, occupied by the epithelial cells within this length (excluding the keratin layer), and the perimeter, *P*, of this area were then recorded. A similar procedure was adopted for measurements on transverse sections. Two sets of area and perimeter measurements were made on each section, and means for each mouse calculated from the total of 6 (from 3 sagittal sections) or 4 (from 2 transverse sections) area and perimeter measurements. Based on these mean values, the mean thickness of epithelium for each mouse was expressed as A/L and the degree of papillary and rete-peg differentiation as the ratio P/L. Simple *t*-tests were then used to make comparisons between the overall means of A/L or P/L values of anaemic mice and their normal littermate controls for the two regions and planes of section of the tongue.

The results (Table 1) indicate that, in the midline of the anterior dorsum of the tongue, the epithelium of anaemic mice was significantly thinner and less well-differentiated into papillae and rete pegs than normal, although for the posterior dorsum neither effect could be demonstrated. It could be argued that the observed difference might have reflected an overall difference of body size between normal and anaemic mice (the present anaemic males weighed, on average, 93 per cent of their littermate controls). However, the fact that lingual epithelium was not universally reduced in

Table 1. Comparison of overall means for average epithelial thickness (A/L) and for epithelial perimeter to length ratio (P/L) for two regions of the tongue in normal ($+/Y$) and anaemic (sla/Y) mice (n = number of mice, p = probability)

	n		A/L (μm)			P/L		
	$+/Y$	sla/Y	$+/Y$	sla/Y	p	$+/Y$	sla/Y	p
Anterior dorsum (sagittal)	28	16	98.8	86.5	<0.02	2.48	2.39	≈ 0.05
Posterior dorsum (transverse)	20	19	71.4	77.7	—	2.25	2.27	—

thickness suggests that this is unlikely to have been the case. The findings are therefore consistent with the tendency towards reduction in thickness of epithelium and loss of filiform papillae from the dorsum of the tongue observed in human iron-deficiency anaemia (Monto, Rizek and Fine, 1961; Jacobs, 1960, 1971), and suggest that the mouse-mutant sex-linked anaemia may be a useful experimental model in which to study more detailed effects of iron deficiency on the oral mucosa. Nevertheless, it should be borne in mind that the effect of the mutant gene on lingual epithelium may be a direct one rather than secondary to the iron-deficiency anaemia.

REFERENCES

Bannerman R. M. and Pinkerton P. H. 1967. X-linked hypochromic anaemia of mice. *Br. J. Haemat.* **13,** 1000–1013.

Edwards J. A. and Bannerman R. M. 1970. Hereditary defect of intestinal iron transport in mice with sex-linked anemia. *J. clin. Invest.* **49,** 1869–1871.

Edwards J. A., Hoke J. E., Mattioli M. and Reichlin M. 1977. Ferritin distribution and synthesis in sex-linked anemia. *J. Lab. clin. Med.* **90,** 68–76.

Jacobs A. 1960. The buccal mucosa in anaemia. *J. clin. Path.* **13,** 463–468.

Jacobs A. 1971. The effect of iron deficiency on the tissues. *Geront. clin.* **13,** 61–68.

Kingston P. J., Bannerman C. E. M. and Bannerman R. M. 1978. Iron deficiency anaemia in newborn *sla* mice: a genetic defect of placental iron transport. *Br. J. Haemat.* **40,** 265–276.

Manis J. 1971. Intestinal iron-transport defect in the mouse with sex-linked anemia. *Am. J. Physiol.* **220,** 135–139.

Monto R. W., Rizek R. A. and Fine G. 1961. Observations on the exfoliative cytology and histology of the oral mucous membranes in iron deficiency. *Oral Surg.* **14,** 965–974.

Pinkerton P. H. 1968. Histological evidence of disordered iron transport in the X-linked hypochromic anaemia of mice. *J. Path. Bact.* **95,** 155–165.

Sorbie J., Hamilton D. L. and Valberg L. S. 1974. Effect of various factors on iron absorption in mice with X-linked anaemia. *Br. J. Haemat.* **27,** 559–569.

Archs oral Biol. Vol. 27, pp. 497 to 503, 1982
Printed in Great Britain.

0003-9969/82/060497-07$03.00/0

EXPERIMENTAL ORAL INFECTION WITH THE YEAST *CANDIDA ALBICANS* IN MICE WITH OR WITHOUT INHERITED IRON-DEFICIENCY ANAEMIA (*sla*)

J. A SOFAER*, W. P. HOLBROOK†‡ and J. C. SOUTHAM*

University of Edinburgh, *Department of Oral Medicine and Oral Pathology, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR and
†Department of Bacteriology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland, U.K.

Summary—The role of iron deficiency in the development of oral candidosis was investigated using the mouse mutant sex-linked anaemia (*sla*). Susceptibility was assessed in terms of the recovery of organisms, particularly from oral swabs, and histological evidence of infection approximately 10 days after the last exposure to *Candida albicans*. The influence of three factors was studied in mixed groups of normal and anaemic mice: mode of inoculation, treatment with tetracycline and treatment with hydrocortisone. The most susceptible group had received drinking water containing tetracycline (1 mg/ml), hydrocortisone (0.1 mg/ml) and candida (5×10^4 c.f.u./ml for 6 days). Anaemic mice showed a rather higher rate of recovery of organisms and more frequent histological evidence of infection that normal mice in certain groups. Neither of these tendencies was statistically significant alone but, taken together, they suggest that some small difference of susceptibility may exist between normal mice and mice with *sla*. The mouse model could be of value in studying the influence of several other inherited disorders on susceptibility to candidosis.

INTRODUCTION

Iron deficiency is thought to be a predisposing factor in the development of oral candidosis in man, although its mode of influence is in dispute (Klebanoff and Clark, 1978; Odds, 1979). The present study was carried out to determine whether the mouse mutant sex-linked anaemia (*sla*) might be a suitable animal model in which to study the effect of iron deficiency on infection with *Candida albicans*. This mutant has an advantage over previous model systems in showing a consistent level of anaemia without artificial induction through dietary restriction or bleeding.

Mice with *sla* have a defect of iron transport in which intestinal cells take up iron but fail to pass it on normally to the circulation (Pinkerton, 1968; Edwards and Bannerman, 1970; Manis, 1971; Sorbie, Hamilton and Valberg, 1974). The anaemia is hypochromic and microcytic. In animals aged up to 200 days, plasma iron is reduced to about one-third of normal (Edwards *et al.*, 1977) with mean red cell counts about 80 per cent, mean packed-cell volumes 60–80 per cent and mean haemoglobin concentrations 40–80 per cent of control values, the anaemia being most severe in younger animals and tending to moderate with age (Bannerman and Pinkerton, 1967). Iron stores, which can be demonstrated clearly in the spleens of normal mice by Prussian-blue staining, are almost invariably absent in mice with *sla*, although occasionally detectable in trace amounts. The spleens of anaemic mice also tend to be enlarged (Pinkerton, 1968). The basic biochemical defect is not known, but it is unlikely to be restricted to the intestinal mucosa because there is evidence of defective placental transport of iron also (Kingston, Bannerman and Bannerman, 1978).

MATERIALS AND METHODS

Experimental groups

Two experiments were carried out, each seeking to compare colonization and infection by *C. albicans* in different groups of mice, all of which started the experiments with a normal natural flora. In the first experiment, there were three groups: 1A, untreated; 1B, receiving candida by oral inoculation; and 1C, receiving candida by oral inoculation together with tetracycline in the drinking water. In the second experiment, there were two groups: 2A, receiving both candida and tetracycline in the drinking water; and 2B, receiving a combination of candida, tetracycline and hydrocortisone in the drinking water. Throughout both experiments, the mice were maintained 5 to a cage. The schedules for the two experiments are shown in Table 1.

All groups were composed of male offspring from matings between heterozygous females (*sla*/+) and normal males. Matings of this type are expected to produce half anaemic males (*sla*/*Y*) and half normal males (+/*Y*). Ages ranged from 57–99 days at the end of the experiments, when blood was taken for haematological analysis, and various other tissues

‡ Present address: Faculty of Odontology, University of Iceland, Landspitalinn, Reykjavik, Iceland.

Table 1. Experimental schedules

	Experiment 1			Experiment 2	
Day	1A	1B	1C	2A	2B
0	S	S	S T	T	T
5	S	S Ca	S | Ca	|	| H
6			|	Ca	| Ca
7		Ca	Ca	|	|
8			|	Ca	| Ca
9		Ca	Ca	|	|
10			|	Ca	| Ca
12			|	↓	| ↓
13	S	S	S |		|
19			|	S	| S
20	S	S	S↓	↓	↓↓
23	End	End	End	End	End

S, swab; T, tetracycline; Ca, candida; H, hydrocortisone.

were removed for microbiological and histological investigation (see below).

Tetracycline and hydrocortisone

Chlortetracycline as pure 'Aureomycin HCl' powder was obtained from Lederle Laboratories (Gosport, Hants, England). A stock solution was prepared and diluted to 1 mg/ml final concentration in the drinking water of groups 1C, 2A and 2B. This concentration has been shown not to affect water intake in the mouse (Helstrom and Balish, 1979). Hydrocortisone as hydrocortisone sodium succinate (Organon, London, England) was added to the drinking water of group 2B at a final concentration of 0.1 mg/ml.

Exposure to candida

A strain of *C. albicans* isolated from human oral chronic atrophic candidosis was used for both experiments. In the first experiment, each mouse received a single oral inoculum of 10^7 colony-forming units (c.f.u.) on each of three occasions over 5 days (Table 1), making a total oral exposure of 3×10^7 c.f.u. per mouse. Each inoculum was given as a drop of a suspension of 10^8 c.f.u./ml. In the second experiment, the organisms were added to the drinking water to produce a suspension with a final concentration of 5×10^4 c.f.u./ml. Exposure was continuous over a total of 6 days, with a fresh drinking-water suspension substituted every other day (Table 1). Assuming an average water consumption of 5 ml/mouse/day (Bernstein, 1966), this gives a total oral exposure of 1.5×10^6 c.f.u. per mouse, one-twentieth of the total exposure in experiment 1. All inocula and drinking-water suspensions were prepared from dilutions of cultures of the test strain that were grown up for 48 h at 37°C in malt broth (see below). Fresh cultures were used for each day on which inoculation was made or the drinking-water suspension changed; all organisms were in the yeast form.

Tissues for haematological, microbiological and histological examination

At the end of both experiments, each animal was killed by etherization, blood was immediately taken for haematological analysis by aspiration from the inferior vena cava, and the spleen, tongue and stomach were removed. In experiment 1, a small portion of stomach and a faecal pellet from the rectum were cultured separately in malt broth (see below). In both experiments, the spleen, tongue and fore-stomach, with lower oesophagus attached, were fixed in 10 per cent buffered formol saline. In experiment 1, each spleen was weighed immediately after removal (before fixation). In experiment 2, each spleen was weighed after fixation. All fixed tissues were embedded in paraffin wax and sectioned at 5 μm. In experiment 1, tongue sections were cut sagittally in the region of the midline; in experiment 2, sections were cut transversely at the junction of the anterior two-thirds and posterior one-third of the tongue. Longitudinal sections were cut through the lower oesophagus and fore-stomach. Sections of tongue and stomach were stained with haematoxylin and eosin, periodic acid Schiff (PAS) and Grocott silver. Sections of spleen were stained with Perl Prussian blue.

Recovery of candida

At different stages of experiments 1 and 2 (Table 1), swabs were taken from the mouth, held open by sterile tweezers, and immediately plated on malt agar (malt-extract syrup; Boots Pure Drug Co., Nottingham, England; 40 g/l in nutrient agar). A standard technique was used so that approximate comparison of colony counts could be made. The portions of stomach and faecal pellets removed at the end of experiment 1 were cultured in malt broth (malt-extract syrup, 20 g/l, in nutrient broth) at 37°C for 24 h and the cultures subsequently plated on malt agar to detect the presence of yeasts. Incubation of malt-agar plates was at 37°C for 48 h in all cases. Malt agar allows considerable morphodifferentiation of yeasts (L.J.R. Milne, 1981, personal communication) and further confirmatory tests were not felt to be necessary for every specimen. However, germ-tube formation was used to confirm the identity of the yeast in one specimen of each batch examined.

RESULTS

Distinguishing between normal and anaemic mice

Automated red-cell counts, and haemoglobin and haematocrit estimates showed that the only one of these parameters for which two non-overlapping distributions of mice could be distinguished was red-cell count. However, due to abnormalities of red cell size in the mutants, some of the count results were difficult to interpret. Haemoglobin and haematocrit values showed bimodal distributions but, because of overlap, it was not possible to use these values to classify individual mice as either normal or anaemic with certainty. Spleen histology almost completely discriminated between mice with definite Prussian-blue staining and those with none. Only a few spleens showed possible trace amounts and these were excluded from the classification. In all other cases, the results of spleen histology were consistent with red-cell count when this was considered reliable. Individual mice could not be classified as normal or anaemic on the basis of spleen weight. The final classification

Table 2. Numbers of normal and anaemic mice in the different experimental groups

Group	Normal	Anaemic	Un-classified	Total
1A	12	8	0	20
1B	9	8	3	20
1C	14	4	2	20
2A	22	22	1	45
2B	18	26	1	45

as either normal or anaemic was therefore based on Prussian-blue staining of the spleens, with confirmatory evidence from haematological investigations when this was available. The numbers of normal and anaemic mice in the different groups are shown in Table 2.

White-cell counts

The mean white-cell counts in group 1A ($9.65 \times 10^9/1$) and group 1C ($9.42 \times 10^9/1$), both inoculated with candida, were significantly higher than in the untreated group 1A ($7.34 \times 10^9/1$; $p \simeq 0.01$ and 0.02 respectively by t tests). White-cell counts were markedly depressed in group 2B (hydrocortisone-treated) compared with group 2A, with an accompanying reduction in spleen weight (Text Fig. 1).

Microbiology

In experiment 1, *C. albicans* was not isolated from any mouse sampled on days 0 or 5. No mouse in group 1A (untreated) yielded candida from any subsequent swab. The recovery of candida from mice in groups 1B and 1C is summarized in Table 3. At day 13, the proportion of mice yielding candida in group 1C (chlortetracycline-treated), and the average colony count for positive isolations, were higher than in group 1B (no chlortetracycline). At days 20/23, no significant difference between groups 1B and 1C could be demonstrated for mouth, stomach or faecal pellet sources individually, although taken together group

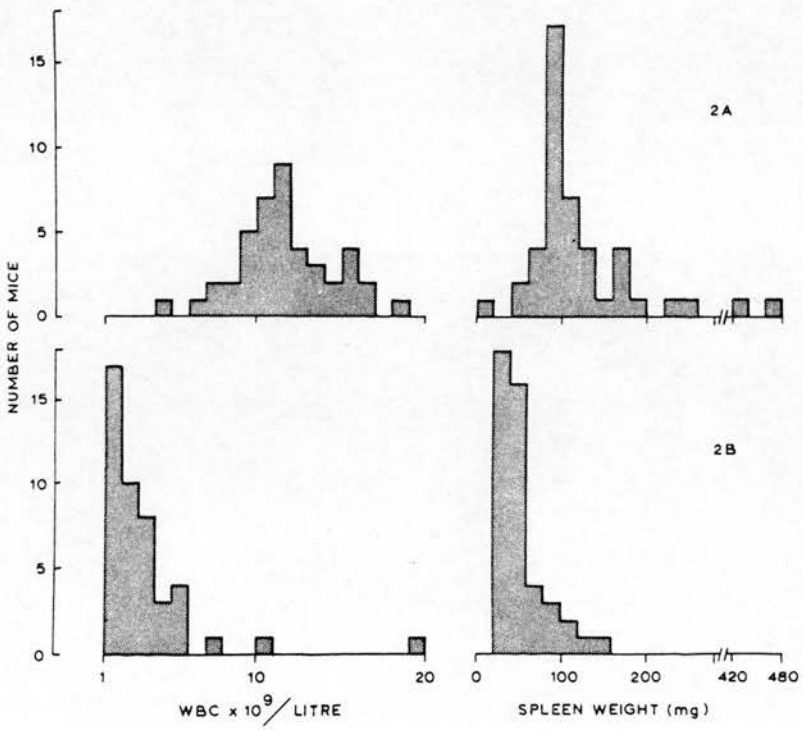


Fig. 1. White-cell counts and spleen weights in experiment 2.

Table 3. Recovery of *C. albicans* on days 13 and 20/23 in experiment 1

	Day 13		Days 20/23			
			+ve isolations out of 20			
	+ve isolations out of 20	Average colony count for +ve isolations	Mouth	Stomach	Faecal pellet	Total +ve (out of 60)
Group 1B	14	14	9	7	10	26
Group 1C	19	30	14	13	14	41
	$p < 0.05$	$p < 0.02$	NS	NS	NS	$p < 0.01$

Significance for +ve isolations by chi-square tests. Significance for colony count difference by t-test after log transformation.

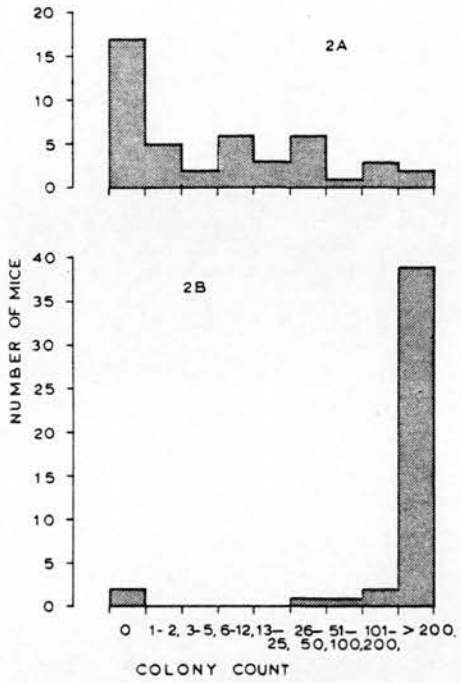


Fig. 2. Recovery of *C. albicans* on day 19 in experiment 2.

1C again showed a significantly higher proportion of positive isolations. There was no significant difference in the isolation of candida between normal and anaemic mice.

In experiment 2, colony counts from swabs taken at day 19 showed a marked enhancing effect of hydrocortisone, almost all mice in group 2B (hydrocortisone-treated) having counts greater than 200 c.f.u. (Text Fig. 2). In group 2A, there was a tendency for higher colony counts among anaemic as opposed to normal mice (Table 4), but this difference was not statistically significant.

Histology

In experiment 1, there was no histological evidence of candidal infection of the tongue, oesophagus or stomach mucosa, although organisms were grown from oral and stomach cultures.

In experiment 2, two types of candidal infection could be demonstrated histologically. In the first, large numbers of candida yeast forms and hyphae penetrated the keratin, but there was no associated inflammatory reaction (Plate Fig. 3a). This type of infection was seen on the dorsal tongue, rarely on the ventral tongue and occasionally in the oesophagus. A prominent pattern on the dorsal tongue surface was one with valleys between the filiform papillae heavily infected, but without involvement of the papillae themselves (Fig. 3b). In the second type of infection, the individual foci were less extensive, the keratin was disrupted, and there was a dense neutrophil-leucocyte infiltrate, either localized to the disrupted keratin (Fig. 3c) or extending through the full thickness of the epithelium (Fig. 3d). Only odd yeast forms and hyphae could be identified in the keratin of these lesions, which were mostly on the dorsal tongue surface but also occasionally in the oesophagus.

The frequencies with which the two different types of infection were found are summarized in Table 5. No difference in type or frequency of infection was found between normal and anaemic mice, except for a tendency towards a higher overall infection rate in the tongues of anaemic compared with normal mice in group 2B (Table 6), although this difference was not statistically significant. Both types of infection were often found in the same mouse.

DISCUSSION

Candida albicans does not appear to contribute to the natural flora of the mouse (Phillips and Balish, 1966). Consistent with this finding was the failure to isolate candida from any swab on days 0 and 5 in experiment 1. Colonization and infection must therefore be induced by varying the experimental conditions if a suitable model for human oral candidosis is to be developed.

Tetracycline

The apparent enhancing effect of tetracycline on candidal infection in man is well known (Odds, 1979). It has been demonstrated in studies of the rat (Russell and Jones, 1973b; Jones *et al.*, 1976) and mouse (Helstrom and Balish, 1979), and in the present study by the difference between groups 1B and 1C (Table 3). There is considerable experimental evidence to indicate that the effect results from suppression of sensitive competing flora (Phillips and Balish, 1966; Nishikawa *et al.*, 1969; Liljemark and Gibbons, 1973; Jones *et al.*, 1976; Helstrom and Balish, 1979), although more direct influences of antibiotic and other treatments on the body's capacity for fungal killing have been reported (Harkness, Grant and Cockle, 1979).

Mode of inoculation

There was no detectable difference in the recovery of candida from oral swabs on days 19/20 between group 1C (3 single inoculations over 5 days) and group 2A (continuous exposure over 6 days). The number of positive isolations from group 1C was 14/20 (Table 3) and from group 2A was 28/45 (Fig. 2). However, whereas histological evidence of infection was absent in group 1C, it was present in a proportion of mice from group 2A (Table 5), even though the total exposure to candida in terms of number of colony-forming units was much lower. The lack of histological evidence of infection in experiment 1 is consistent with the reduction in number of positive isolations from oral swabs between days 13 and 20, both for groups 1B and 1C (Table 3). This suggests predominance of colonization rather than infection.

Table 4. Numbers of normal and anaemic mice in group 2A showing three different levels of colony count from oral swabs taken on day 19

	Colony count		
	0	1–25	>25
Normal	10	10	2
Anaemic	7	6	9

Table 5. Numbers of mice (each out of a total of 45) showing the two different types of infection

	Tongue		Oesophagus	
	Y & H	Polys	Y & H	Polys
Group 2A	1	8	1	2
Group 2B	36	19	22	4

Y & H = infection with yeast forms and hyphae predominating. Polys = infection with tissue reaction predominating.

gradual elimination of yeasts occurring with time after the most recent inoculation, as reported by Helstrom and Balish (1979). Nevertheless, some degree of undetected penetration of candida into the tissues seems likely because the white-cell count was significantly elevated in groups 1B and 1C.

Hydrocortisone

The most striking finding was the effect on susceptibility produced by treatment with hydrocortisone (Fig. 2; Table 5). The enhancing effect of steroids on candidal infection has been noted both in man (Odds, 1979) and experimental animals (Louria, Fallon and Browne, 1960; Cantrell and Widra, 1964; Hurley, Balow and Fauci, 1975). Systemic steroids have a wide range of effects on the body, including the suppression of both inflammatory and cell-mediated immune responses (Haynes and Murad, 1980). This perhaps accounts for the relatively infrequent tissue response in group 2B (hydrocortisone-treated) compared with group 2A, although both types of infection were found in both groups (Table 5). Steroid treatment also produces a lymphocytopenia and, in rodents, rapid and dramatic dissolution of lymphoid tissue (Haynes and Murad, 1980). Mice have a much higher proportion of circulating lymphocytes (65–75 per cent; Russell and Bernstein, 1966) than man (20–25 per cent), so the observed leucopenia and reduction in spleen weight (Fig. 1) can be attributed largely to the general effect of hydrocortisone on the lymphoid system.

Suitability of the model

Histologically, the pattern of infection produced was similar to that observed in human oral candidosis, though the reason why a tissue response should be found in some instances but not in others is not clear. A recent report suggests that strain variation of *C. albicans* may be a factor determining whether or not a tissue response occurs (Field *et al.*, 1981).

Table 6. Numbers of normal and anaemic mice in group 2B in which histological evidence of infection of the tongue was absent (−ve) or present (+ve). Both types of infection are included

	−ve	+ve
Normal	5	13
Anaemic	2	24

There were two independent and consistent tendencies among anaemic mice in experiment 2, one towards increased recovery of candida from group 2A (Table 4) and the other towards increased infection, irrespective of type, in group 2B (Table 6). Although neither of these was statistically significant individually, taken together they suggest that some small difference in susceptibility to candida may exist between normal mice and mice with *sla*. Any increased susceptibility of these anaemic mice could be related to their thinner lingual epithelium (Steele, Sofaer and Southam, 1981), which is consistent with the tendency towards reduction in thickness of epithelium and loss of filiform papillae from the dorsum of the tongue observed in human iron-deficiency anaemia (Monto, Rizek and Fine, 1961; Jacobs, 1960, 1971). It should, however, be borne in mind that any effect of the mutant gene on the mucosa could possibly be direct rather than secondary to the iron deficiency.

More generally, a mouse model has advantages over the rat model in which a number of studies of oral candidosis have already been made (Jones and Adams, 1970; Adams and Jones, 1971; Jones and Russell, 1973; Russell and Jones, 1973a,b; Jones *et al.*, 1976). First, mice are cheaper to house and maintain, so that larger numbers can be used at lower cost; secondly, several mutants are available to provide consistent and repeatable variants, many apparently homologous with human disorders, that could usefully be employed in the study of candidosis. In addition to other anaemic mutants (Bannerman and Edwards, 1976; Harrison, 1979), there are endocrine disorders such as diabetes (Hummel, Dickie and Coleman, 1966) and immune disorders such as the athymic state, already used in the study of candidosis (Helstrom and Balish, 1979), and the more recently described X-linked B lymphocyte defect (Scher *et al.*, 1980). A mouse model could also be of value for more detailed studies of the role of antibiotics and steroids in susceptibility to candidosis, for the testing of anticandidal drugs, and for the evaluation of candidal strain infectivity.

REFERENCES

Adams D. and Jones J. H. 1971. Life history of experimentally induced oral candidiasis in the rat. *J. dent. Res.* **50**, 643–644.

Bannerman R. M. and Edwards J. A. 1976. Hereditary anaemias in laboratory animals. *Br. J. Haemat.* **32**, 299–307.

Bannerman R. M. and Pinkerton P. H. 1967. X-linked hypochromic anaemia of mice. *Br. J. Haemat.* **13**, 1000–1013.

Bernstein S. E. 1966. Physiological characteristics. In: *Biology of the Laboratory Mouse*, 2nd edn (Edited by Green E. L.) Chap. 16, pp. 337–350. McGraw-Hill, New York.

Cantrell H. R. and Widra A. 1964. Experimental candidiasis in cortisone treated mice. *J. Bact.* **87**, 1532.

Edwards J. A and Bannerman R. M. 1970. Hereditary defect of intestinal iron transport in mice with sex-linked anemia. *J. clin. Invest.* **49**, 1869–1871.

Edwards J. A., Hoke J. E., Mattioli M. and Reichlin M. 1977. Ferritin distribution and synthesis in sex-linked anemia. *J. Lab. clin. Med.* **90**, 68–76.

Field L. H., Pope L. M., Cole G. T., Guentzel M. N. and Berry L. J. 1981. Persistence and spread of *Candida albi-*

cans after intragastric inoculation of infant mice. *Infect. Immun.* **31**, 783–791.

Harkness R. A., Grant M. and Cockle S. M. 1979. Modification of genetic expression in phagocytes. In: *Inborn Errors of Immunity and Phagocytosis* (Edited by Güttler F., Seakins J. W. T. and Harkness R. A.) Chap. 20, pp. 297–307. MTP Press, Lancaster, England.

Harrison D. E. 1979. Use of genetic anaemias in mice as tools for haematological research. *Clin. Haemat.* **8**, 239–262.

Haynes R. C. Jr and Murad F. 1980. Adrenocorticotropic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of adrenocortical steroid biosynthesis. In: *The Pharmacological Basis of Therapeutics*, 6th edn (Edited by Gilman A. G., Goodman L. S. and Gilman A.) Chap. 63, pp. 1466–1496. Macmillan, New York.

Helstrom P. B. and Balish E. 1979. Effect of oral tetracycline, the microbial flora, and the athymic state on gastrointestinal colonization and infection of BALB/c mice with *Candida albicans*. *Infect. Immun.* **23**, 764–774.

Hummel K. P., Dickie M. M. and Coleman D. L. 1966. Diabetes, a new mutation in the mouse. *Science* **153**, 1127–1128.

Hurley D. L., Balow J. E. and Fauci A. S. 1975. Experimental disseminated candidiasis. II. Adminstration of glucocorticosteroids, susceptibility to infection, and immunity. *J. infect. Dis.* **132**, 393–398.

Jacobs A. 1960. The buccal mucosa in anaemia. *J. clin. Path.* **13**, 463–468.

Jacobs A. 1971. The effect of iron deficiency on the tissues. *Geront. clin.* **13**, 61–68.

Jones J. H. and Adams D. 1970. Experimentally induced acute oral candidosis in the rat. *Br. J. Derm.* **83**, 670–673.

Jones J. H. and Russell C. 1973. Experimental oral candidiasis in weanling rats. *J. dent. Res.* **52**, 182.

Jones J. H., Russell C., Young C. and Owen D. 1976. Tetracycline and the colonization and infection of the mouths of germ-free and conventionalized rats with *Candida albicans*. *J. Antimicrob. Chemother.* **2**, 247–253.

Kingston P. J., Bannerman C. E. M. and Bannerman R. M. 1978. Iron deficiency anaemia in newborn *sla* mice: a genetic defect of placental iron transport. *Br. J. Haemat.* **40**, 265–276.

Klebanoff S. J. and Clark R. A. 1978. *The Neutrophil: Function and Clinical Disorders*, pp. 570–571. North-Holland, Amsterdam.

Liljemark W. F. and Gibbons R. J. 1973. Suppression of *Candida albicans* by human oral streptococci in gnotobiotic mice. *Infect. Immun.* **8**, 846–849.

Louria D. B., Fallon N. and Browne H. G. 1960. The influence of cortisone on experimental fungus infections in mice. *J. clin. Invest.* **39**, 1435–1449.

Manis J. 1971. Intestinal iron-transport defect in the mouse with sex-linked anemia. *Am. J. Physiol.* **220**, 135–139.

Monto R. W., Rizek R. A. and Fine G. 1961. Observations on the exfoliative cytology and histology of the oral mucous membranes in iron deficiency. *Oral Surg.* **14**, 965–974.

Nishikawa T., Hatano H., Ohnishi N., Sasaki S. and Nomura T. 1969. Establishment of *Candida albicans* in the alimentary tract of the germ-free mice and antagonism with *Escherichia coli* after oral inoculation. *Jap. J. Microbiol.* **13**, 263–276.

Odds F. C. 1979. *Candida and Candidosis*. Leicester University Press, Leicester.

Phillips A. W. and Balish E. 1966. Growth and invasiveness of *Candida albicans* in the germ-free and conventional mouse after oral challenge. *Appl. Microbiol.* **14**, 737–741.

Pinkerton P. H. 1968. Histological evidence of disordered iron transport in the X-linked hypochromic anaemia of mice. *J. Path. Bact.* **95**, 155–165.

Russell C. and Jones J. H. 1973a. The effects of oral inoculation of the yeast and mycelial phases of *Candida albicans* in rats fed on normal and carbohydrate rich diets. *Archs oral Biol.* **18**, 409–412.

Russell C. and Jones J. H. 1973b. Effects of oral inoculation of *Candida albicans* in tetracycline-treated rats. *J. med. Microbiol.* **6**, 275–279.

Russell E. S. and Berstein S. E. 1966. Blood and blood formation. In: *Biology of the Laboratory Mouse*, 2nd edn (Edited by Green E. L.) Chap. 17, pp. 351–372. McGraw-Hill, New York.

Scher I., Berning A. K., Kessler S. and Finkelman F. D. 1980. Development of B lymphocytes in the mouse; studies of the frequency and distribution of surface IgM and IgD in normal and immune defective CBA/N F_1 mice. *J. Immun.* **125**, 1686–1693.

Sorbie J., Hamilton D. L. and Valberg L. S. 1974. Effect of various factors on iron absorption in mice with X-linked anaemia. *Br. J. Haemat.* **27**, 559–569.

Steele B., Sofaer J. A. and Southam J. C. 1981. Lingual epithelial thickness in mice with inherited iron-deficiency anaemia (*sla*). *Archs oral Biol.* **26**, 343–344.

Plate 1.

Fig. 3a. Mouse dorsum tongue (Group 2B) with numerous candida yeast forms and hyphae penetrating the keratin, but with no associated inflammatory reaction. PAS. × 222.

Fig. 3b. Mouse dorsum tongue (Group 2B) showing candida yeast forms and hyphae restricted to the valleys between the filiform papillae. PAS. × 222.

Fig. 3c. Mouse dorsum tongue (Group 2B) with neutrophil-leucocyte infiltration in the disrupted surface keratin. Haematoxylin and eosin. × 222

Fig. 3d. Mouse dorsum tongue (Group 2B) showing dense neutrophil-leucocyte infiltration extending through the whole thickness of the epithelium with disruption of the surface keratin. Haematoxylin and eosin. × 133

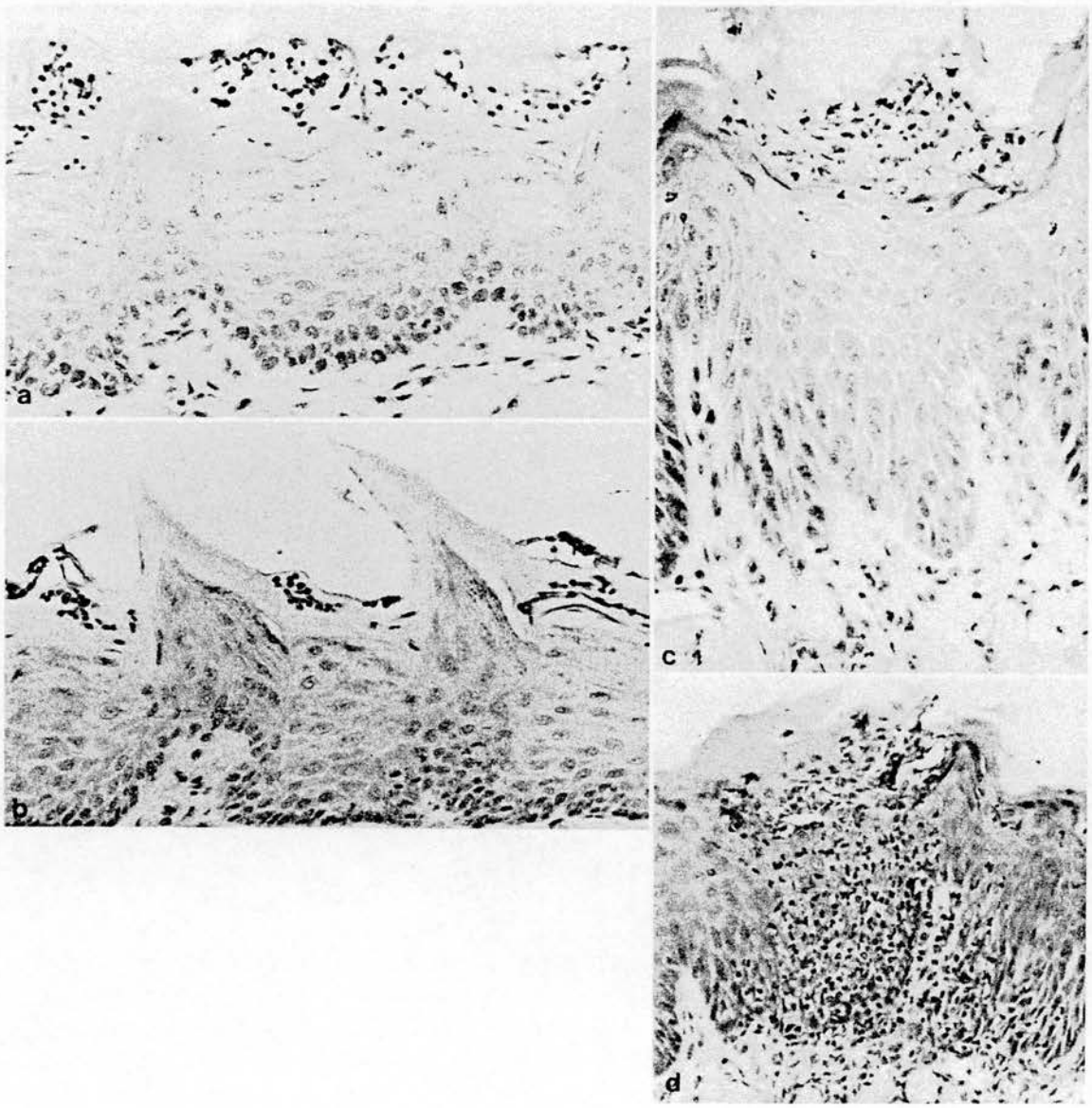


Plate 1.

EXPERIMENTAL ORAL INFECTION OF MICE WITH A PATHOGENIC AND A NON-PATHOGENIC STRAIN OF THE YEAST *CANDIDA ALBICANS*

W.P. Holbrook*, J.A. Sofaer† and J.C. Southam†

Running title:

Experimental oral infection by two candida strains

* University of Iceland,
Faculty of Odontology,
Landspitalinn, Reykjavik,
Iceland

and

† University of Edinburgh,
Department of Oral Medicine and Oral Pathology,
Old Surgeons Hall, High School Yards,
Edinburgh EH1 1NR

Summary - Oral infection by Candida albicans is thought to be related more to host susceptibility than to virulence of the organism. However, using a simple mouse model of oral candidosis, differences in colonisation and infection between two strains of C. albicans have been demonstrated.

INTRODUCTION

In humans, oral infection by the yeast Candida albicans is thought to be primarily related to host susceptibility, and several predisposing factors are well known. These include the wearing of dentures, iron deficiency anaemia, diabetes, debilitation, and several drugs such as steroids, immunosuppressives and broad spectrum antibiotics. Little attention has been given to the possible association between virulence and the capacity of the organism to cause oral candidosis, although variation in pathogenicity of C. albicans by other criteria has occasionally been reported (Louria, Brayton and Finkel, 1963; Albano and Schmitt, 1973; Richardson and Smith, 1980). The purpose of the investigation reported here was to test the hypothesis that the ability to cause oral candidosis is partly related to virulence using the experimental mouse model recently described (Sofaer, Holbrook and Southam, 1982), a system representative of the human situation but uncomplicated by individual variation in host susceptibility.

MATERIALS AND METHODS

Two groups of 40 male mice from the inbred strain CBA/ca (Centre for Laboratory Animals, University of Edinburgh) were used, 8 cages of 5 mice per cage for each group. Ages ranged from 45 to 121 days at the start of the experiment, with the two groups matched

with respect to age distribution. All mice were given drinking water containing 1 mg/ml chlortetracycline throughout the experiment (pure 'Aureomycin HCl' powder from Lederle Laboratories, Gosport, Hants, England), and 0.1 mg/ml hydrocortisone from day 5 until the end of the experiment (hydrocortisone sodium succinate from Organon, London, England). No gross differences in water consumption between mice were noted (Helstrom and Balish, 1979; Sofaer et al., 1982). Doses received by the mice were approximately 200 mg/kg/day of chlortetracycline and 20 mg/kg/day of hydrocortisone.

Two strains of C. albicans were obtained from Professor H. Smith, Department of Microbiology, University of Birmingham, England. One (strain 19321) had been isolated from a case of vaginal thrush and was termed virulent, while the other (strain 22114) had come from a 'transient candidaemia' and was termed attenuated, although virulence had not been artificially reduced. The virulent strain adhered more readily to buccal epithelium (Kearns, Richardson and Smith, 1980) and showed greater resistance to intracellular killing by phagocytes (Richardson and Smith, 1980) than the attenuated strain. Both strains have been independently identified as C. albicans (T. Plácido, Curator, Culture Collection, Laboratorio de Microbiologia, Instituto Gulbenkian de Ciência, Oeiras, Portugal, 1981, personal communication). The strains were grown up in malt broth (malt extract syrup - Boots Pure Drug Co., Nottingham, England - 20 g/l in nutrient broth) overnight and added to the drinking water on day 6 at a final concentration of 10^4 colony-forming units per ml. The virulent strain was given to one group of mice and the attenuated strain to the other. Exposure to the organisms was continuous at this level

over a total of 6 days, with a fresh drinking water suspension substituted every other day. From day 12 all mice were given drinking water containing chlortetracycline and hydrocortisone only.

Oral swabs were taken at the start of the experiment and again on day 19. The mouth of each animal was held open with sterile forceps and a small sterile cotton wool pledget, held in fine sterile tweezers and moistened with malt broth, was wiped over the dorsum of the tongue and the buccal and palatal mucosa. Swabs were plated immediately on malt agar (malt extract syrup 40 g/l in nutrient agar) using a standard plating technique so that approximate comparisons of colony counts could be made. Colony counting was carried out after incubation of the plates at 37^0C for 48 h.

All mice were killed by etherisation on day 21. The tongues were removed, fixed in 10 per cent buffered formol saline, embedded in paraffin wax and sectioned at 5 μm transversely at the junction of the anterior two-thirds and posterior one-third of the tongue. Sections were stained with haematoxylin and eosin, periodic acid Schiff and Grocott silver.

RESULTS

The two strains of C. albicans were somewhat different from each other in colonial appearance when grown on malt agar, but considerable differences were seen in pseudohyphal production when grown on corn-meal agar. The virulent strain (19321) produced a small number of short pseudohyphae and very few

chlamydospores while the attenuated strain (22114) produced abundant long pseudohyphae and large numbers of chlamydospores. Both strains were germ-tube positive and showed the carbohydrate assimilation pattern of C. albicans, the only difference from the standard description being in the assimilation of xylose which was negative for the attenuated strain. The biochemical and cultural reactions of the two strains are summarised in Table 1.

No yeasts were isolated from oral swabs taken at the start of the experiment. The colony counts from swabs taken on day 19, 7 days after the last exposure to candida in the drinking water, are shown in Fig. 1. There was a clear difference in the distribution of colony counts between strains.

Two types of histological appearance were seen in the tongue sections as already described and illustrated (Sofaer et al., 1982). In the first, large numbers of blastospores and hyphae penetrated the keratin but there was no associated inflammatory reaction. In the second, there was disruption of the keratin with a dense polymorphonuclear leucocyte infiltration, either localised to the disrupted keratin or extending through the full thickness of the epithelium. The relative frequencies of these two types of appearance in mice exposed to the different candida strains are summarised in Table 2. Comparisons by chi square test showed that for the virulent strain, over all colony counts, the inflammatory response was found significantly more often than blastospores and hyphae alone (33:7 compared with 4:36, $p < 0.01$), while for the attenuated strain the inflammatory response was found significantly more often with higher than with lower counts (12:8 compared with

4:16, $p < 0.05$). Comparisons between strains revealed that, over all colony counts, the virulent strain produced the inflammatory type of response significantly more often than the attenuated strain (33:7 compared with 16:24, $p < 0.01$).

DISCUSSION

Variation in pathogenicity between strains of C. albicans has received only limited attention, particularly with regard to the capacity to produce oral candidosis. Louria et al. (1963) showed that a strain of C. albicans isolated from the pharynx of a patient with chronic mucocutaneous candidosis was much less virulent than strains isolated from the blood of 5 patients with disseminated candidosis. Their test involved an intravenous injection of 5×10^6 blastospores into mice, with death rate and histological signs of renal damage being used as criteria for virulence. Albano and Schmitt (1973) carried out a similar study and showed that one strain of C. albicans isolated from the mouth of a patient on long-term antibiotics was less virulent than two strains isolated as sole pathogens from fatal burns cases. As evidence for differences in virulence of C. albicans strains in the mouth, Martin and Lamb (1982) reported that 29 of 30 patients with denture stomatitis yielded C. albicans serotype A only from the lesions, although mixed serotypes were found in unaffected parts of the same mouths. Lesions produced by subcutaneous injection of C. albicans into rats were more extensive for strains isolated from a case of

denture stomatitis than for those isolated from healthy carriers (Abdelghaffer and Russell, 1979).

The two strains used in the present study fall within the currently accepted definition of the species C. albicans. Within the attenuated strain there was an association between colony count and histological evidence of infection. Differences between strains were found both in the level of oral colonisation and for the ability to cause disruption of the keratin and an inflammatory response. The present in vivo findings therefore complement the earlier in vitro studies on the two strains (Richardson and Smith, 1980; Kearns et al., 1980) and are consistent with the recent suggestion that strain variation in C. albicans may be a factor determining whether or not a tissue response occurs (Field et al., 1981). The present study has demonstrated that strains of C. albicans may differ in their capacity to produce infection in an experimental system more closely representative of human oral candidosis than those that have used lethality or the response to infection in other sites as the criterion for virulence.

Table 1. Biochemical and cultural reactions of the two *C. albicans* strains. (See Odds, 1979)

Test	Virulent (19321)	Attenuated (22114)
Assimilation of:		
Glucose	+	+
Sucrose	+	+
Lactose	-	-
Trehalose	+	+
Rhamnose	+	+
Xylose	+	-
Surface growth	-	-
Urease	-	-
Pseudohyphae	few, short	abundant, long
Chlamydospores	few	many

Table 2. Numbers of mice negative and positive for histological evidence of infection of the dorsum of the tongue, by colony count.

		Blastospores and hyphae		Polymorph infiltration	
	Colony count	-ve	+ve	-ve	+ve
Virulent (19321)	Low ($\leq$ 50)	21	1	6	16
	High ($\geq$ 51)	15	3	1	17
	All counts	36	4	7	33
Attenuated (22114)	Low ($\leq$ 12)	18	2	16	4
	High ($\geq$ 13)	12	8	8	12
	All counts	30	10	24	16

Footnote:

The classification of mice into 'low' and 'high' colony count groups was to provide approximately equal groups within each strain. The difference in the classification between strains means that comparisons between 'low' and 'high' groups are only valid within strains.

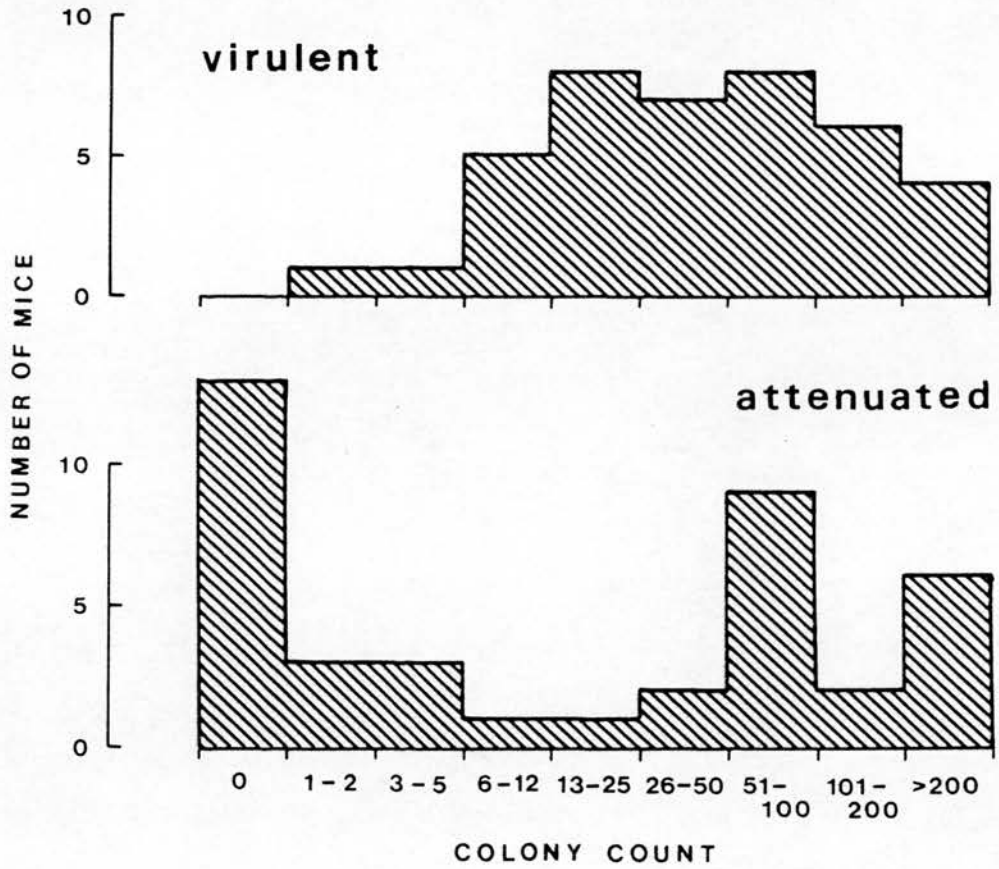


Fig. 1. The distribution of colony counts from oral swabs taken on day 19.

References

Abdelghaffer A.A. and Russell C. 1979. The role of strain difference and tetracycline on the production of subcutaneous lesions by Candida albicans in experimental rats. Sabouraudia 17, 210-217.

Albano M.M. and Schmitt J.A. 1973. Pathogenicity in mice of strains of Candida albicans (Robin) Berk. isolated from burns patients. Mycopathol. Mycol. Appl. 49, 283-289.

Field L.H., Pope L.M., Cole G.T., Guentzel M.N. and Berry L.J. 1981. Persistence and spread of Candida albicans after intragastric inoculation of infant mice. Infect. Immun. 31, 783-791.

Helstrom P.B. and Balish E. 1979. Effect of oral tetracycline, the microbial flora and the athymic state on gastrointestinal colonization and infection of BALB/C mice with Candida albicans. Infect. Immun. 23, 764-774.

Kearns M.J., Richardson M.D. and Smith H. 1980. Adhesion of Candida albicans to buccal epithelium: differential behaviour of virulent and attenuated strains. Soc. Gen. Microbiol. Quart. 7, 131-132.

Louria D.B., Brayton R.G. and Finkel G. 1963. Studies on the pathogenesis of experimental Candida albicans infections in mice. Sabouraudia 2, 271-283.

Martin M.V. and Lamb D.J. 1982. Frequency of Candida albicans serotypes in patients with denture-induced stomatitis and in normal denture wearers. J. Clin. Pathol. 35, 888-891.

Odds F.C. 1979. Candida and Candidosis. Leicester University Press, Leicester.

Richardson M.D. and Smith H. 1980. The interaction of attenuated and virulent strains of Candida albicans with human and mouse phagocytes. Soc. Gen. Microbiol. Quart. 7, 131.

Sofaer J.A., Holbrook W.P. and Southam J.C. 1982. Experimental oral infection with the yeast Candida albicans in mice with or without inherited iron-deficiency anaemia (sla). Archs. oral Biol. 27, 497-503.

A FAMILY STUDY OF PAGET'S DISEASE OF BONE

J.A. Sofaer[*†], S.M. Holloway[†] and A.E.H. Emery[†]

[*] Department of Oral Medicine and Oral Pathology,
Old Surgeons Hall, High School Yards,
Edinburgh EH1 1NR

and

[†] University Department of Human Genetics,
Western General Hospital,
Edinburgh EH4 2XU

SUMMARY - Familial aggregation of Paget's disease of bone occurs occasionally and an exclusively genetic aetiology has been proposed in the past. On the other hand, epidemiological surveys point to an important environmental contribution, and evidence is accumulating to suggest that the disease may be caused by a slow virus infection. Analysis of 407 family history questionnaires completed by Paget's disease patients confirmed the familial nature of the disease. Overall, the findings were consistent with the hypothesis that Paget's disease is caused by infection with a common and widespread virus superimposed on genetic variation for susceptibility and perhaps severity of the disease.

Paget's disease of bone is a remarkably common condition in later life, radiological surveys having detected characteristic bony changes in 2.3 - 8.3 per cent of the British population at age 55 years and above, with a moderate male predominance (σ/φ = 1.59) (1). The disease involves rapid remodelling and the development of structurally abnormal bone, and can be the cause of pain, fracture deformity and rarely malignancy. Many affected individuals are asymptomatic, but because of its high prevalence the disease still makes a considerable contribution to morbidity among the elderly (2).

Paget's disease shows a degree of familial aggregation, including occurrence in successive generations, and has consequently been listed as a possible autosomal dominant disorder (3). Genetic linkage has also been suggested between a presumed Paget's disease locus and the HLA complex on chromosome 6 (4). On the other hand, there is increasing evidence of an important environmental contribution. For example, prevalence among migrants from the United Kingdom to Australia, although higher than for native Australians, is lower than for British residents (5); and within Britain there is a remarkably localised area of particularly high prevalence in Lancashire (1). In the United States, blacks and whites show similar prevalences that vary from one part of the country to another, even though the disease is rare among African blacks (6).

The most exciting recent finding is ultrastructural and immunohistological evidence of viruses within the osteoclasts of affected bone, suggesting that Paget's disease may be caused by a slow virus infection (7). Two different viruses have been implicated, measles virus (8) and respiratory syncytial virus (RSV) (9). This apparent inconsistency is a little puzzling, although very recent

work suggests that the two viruses may have an antigen in common (10). Attempts to demonstrate raised viral antibody titres in the serum of Paget's disease patients have been unsuccessful for measles (11,12) while for RSV there have been conflicting results (12,13).

The present study was undertaken in the hope of gaining further information on the aetiology of Paget's disease. It differs from previous work in that an attempt has been made to analyse both genetic and environmental influences on the disease within the same sample.

SUBJECTS AND METHODS

During 1980 and 1981, 595 family history questionnaires were distributed to known sufferers of Paget's disease, almost all of whom were ascertained through the British National Association for the Relief of Paget's Disease. Questions were asked about the index cases, their first degree relatives, their spouses and their spouses' first degree relatives. For each index case or relative the information requested included: year of birth, sex, place of birth and main place of residence in each of three different age ranges, occupation, any chronic illness other than Paget's disease and, if affected by Paget's disease, the age of onset and any illness or unusual circumstance that occurred within the year prior to onset of the Paget's disease. Respondents were not asked to give details of the circumstances under which each diagnosis of Paget's disease had been made.

Occupations were coded according to the Office of Population Censuses and Surveys 1970 classification (14), and illnesses according to the International Classification of Diseases (15). For the purpose of analysis each known place of birth or residence was specified by one of the 120 outer postcode areas in the United Kingdom (the single or double letter code at the beginning of the complete postcode), or by country if abroad.

RESULTS

Composition of the sample and prevalence among relatives

A total of 407 questionnaires were returned completed, a response rate of 68 per cent, the mean age of index cases being 73 years. The numbers of different types of relative who had survived to at least 55 years, and the numbers affected by Paget's disease, are given in Table 1. The prevalence of the disease among parents and sibs of index cases (57/1450 = 3.93%) was about ten times greater than among parents and sibs of spouses (3/823 = 0.36%), and the same degree of female predominance was found among parents and sibs of index cases ($\varphi/\sigma^{\uparrow}$ = 37/20 = 1.85) as among the index cases themselves ($\varphi/\sigma^{\uparrow}$ = 255/152) = 1.68). There was no significant difference of prevalence between the parents of index cases and the sibs of index cases at age 55 and above. After combining parents and sibs there was no significant difference between male and female index cases in the proportions of these relatives affected. However, male index cases differed from female index cases in that they had significantly

($p < 0.01$) fewer affected male relatives (fathers and brothers) than affected female relatives (mothers and sisters).

Of the 407 index cases, 56 (13.8%) had a family history of Paget's disease. Of these 56 familial cases 31 came from families where successive generations were affected ('dominant') and 25 from families where only sibs were affected ('recessive'). Overall there were 42 concordant parent-offspring pairs, 17 involving fathers (3 of which were father-son pairs) and 25 involving mothers. Among the 33 sibships that contained more than one individual reported to have Paget's disease there were 39 sib pairs concordant and 194 sib pairs discordant for the disease.

Onset of Paget's disease

The mean age of onset (awareness of having the disease) was significantly earlier among familial than among isolated cases (Table 2) but there was no difference between 'dominant' and 'recessive' cases. The within family variance for age of onset was significantly lower than the between family variance, indicating that relatives tended to be more alike with respect to age of onset than unrelated individuals, and the within sibship variance for year of onset was significantly greater than that for age of onset (Table 3).

Only two unusual circumstances occurring within the year preceding onset were each reported by more than one per cent of the total of 480 cases (index cases plus affected relatives), a fall in 23 patients (4.8%) and a road accident in 6 patients (1.3%).

Other chronic illnesses

Index cases were asked if they or their relatives suffered from any chronic illness or disability other than Paget's disease, and if so to name the one that was the most troublesome. The distribution of the most troublesome other chronic illnesses showed a significant difference ($p < 0.001$) between index cases on the one hand and normal living sibs and spouses' sibs on the other, deafness and arthropathy being reported more often among index cases.

Occupation and social class

Male index cases were compared with normal male sibs of cases and female index cases with normal female sibs of cases. For both sexes a greater proportion of index cases than of normal sibs was engaged in non-manual work. The proportions in males were 54% as opposed to 47%, and in females were 71% as opposed to 59%. The difference was significant for females ($p < 0.01$). In particular, there was an excess of index cases in Occupation Unit Groups 24 (Administrators and Managers) and 25 (Professionals, Technical Workers and Artists). Some 26% of male index cases fell into these groups compared with 16% of normal male sibs, and 23% of female index cases fell into these groups compared with 12% of normal female sibs. The difference was significant both for males ($p < 0.01$) and for females ($p < 0.001$). Normal sibs of spouses were also less likely to be engaged in non-manual work and to be in Occupation Unit Groups 24 and 25 than index cases but none of the differences were statistically significant.

The distribution of social class among index cases showed a significant ($p < 0.005$) overrepresentation of the higher classes compared with the general population (16).

Places of birth and residence

Table 4 shows the distribution of concordant and discordant sib pairs from the 33 sibships that each contained more than one case of Paget's disease. The two members of each pair were classified as having been born or having had their main place of residence over three age ranges in either the same or different postcode areas. There was no significant tendency for concordant pairs to have been born or to have lived in the same postcode area and for discordant pairs to have been born or to have lived in different postcode areas. A similar analysis was carried out using all sibships of index cases, comparing those having only a single case of Paget's disease with the 33 multiple case sibships. Sibships were classified as all members born or having lived in either a single postcode area or multiple postcode areas. There was no significant association between single postcode areas and multiple case sibships, either for place of birth or for main place of residence in any of the three age ranges.

Year of birth

Among the index cases there appeared to be a fluctuating distribution of years of birth with a periodicity of around 3 years (Fig. 1a), reminiscent of the cyclic changes in prevalence known to occur for various infectious diseases. Such fluctuations could be the result of cyclic changes of birth rate or differences in longevity associated with different years of birth, independent of Paget's disease. Accordingly, the number of index cases known to have been born in England and Wales in each year was expressed as a

proportion of all persons with the same year of birth in the total population of England and Wales at 31st December 1980. The total population figures used were unpublished estimates provided by the Population Estimates Unit of the Office of Population Censuses and Surveys. Figure 1b shows that after this adjustment the fluctuations in relative frequency of different birth years remain. However, using standard statistical tests no significant cyclic variation for any given periodicity was found in the relative frequency of Paget's disease cases for different years of birth.

DISCUSSION

Genetics

Previous investigations into the genetics of Paget's disease have been of two types: pedigree studies, those concerned with the pattern of distribution of the disease in selected families; and studies of prevalence among relatives of unselected samples of index cases. The pedigree data (17-20) appear to be consistent with dominant inheritance and reduced penetrance. The results of the present study are compatible with this, there being no detectable

difference between 'dominant' and 'recessive' cases. However, there are two main difficulties of interpretation. First there is the variable but generally rather late onset of the disease, and second the lack of uniformity of diagnostic criteria, in particular the absence of symptoms in a large proportion of those who, on radiological evidence, would be regarded as having the disease. Conventional segregation analysis is therefore not appropriate and it seems necessary, at least initially, to resort to simple quantitative expressions of the familial nature of the disease. Table 5 illustrates the wide range in the proportion of cases reported to have had a positive family history. This variation may simply reflect differences in the level of diagnosis.

In the present study the tenfold higher prevalence of Paget's disease among parents and sibs of index cases (3.93%) compared with parents and sibs of spouses (0.36%) both illustrates the familial nature of the disease and gives some indication of the overall level of detection. The prevalence of radiological evidence of the disease at age 55 years and above is around 5 per cent in the United Kingdom (1) so, if parents and sibs of spouses are regarded as representative of the general population, only 7 per cent of those with radiological signs have been detected by the questionnaire. This is in good agreement with the usually quoted figure of 5 per cent for the proportion of affected individuals who have symptoms (27).

The female predominance observed, which occurred both among index cases and among parents and sibs of index cases, appears to conflict with the generally reported male predominance. This finding could perhaps be explained by the more frequent GP consultations of females (28), leading to a higher probability of detection of mild or

asymptomatic cases and therefore a higher degree of self awareness for the disease among females.

The difference between isolated and familial cases for mean age of onset (Table 2) is consistent with different levels of genetic contribution to the two types of case but could also be explained by the likelihood of earlier diagnosis in families already known to contain an affected individual. Likewise, the similarity of age of onset within families (Table 3) could be caused either by common genes or by common environmental influences. However, the greater within sibship variance of year of onset compared with age of onset (Table 3) suggests that onset is influenced more by the length of time that has elapsed since birth than by any environmental factor of short duration affecting members of the same sibship together. This is in keeping with a genetic influence on age of onset, but does not exclude an origin for the disease through the effect of a common and widespread environmental agent acting at or around the time of birth, or of long term environmental effects.

It is possible to estimate the heritability of 'self knowledge of Paget's disease' using the method of Falconer (29), assuming that 'self knowledge' constitutes the more severe extreme of the distribution of those with radiological signs. Barker et al (1) have given the overall British prevalence of radiological signs in those aged 55 years and above as 6.2% for men and 3.9% for women. Assuming that only 5% of those with radiological signs are aware that they have the disease, the population prevalence of 'self knowledge' is then 0.31% for males and 0.20% for females. Using the figures for prevalence among parents and sibs who survived to at least 55 years (Table 1), the heritabilities calculated for 'self knowledge of Paget's disease' are 21±17% for males

and 77±6% for females. The relatively low estimate and high standard error for males are due to the fact that so few male index cases had affected male relatives.

Other factors

The reporting of a fall or a road accident within the year prior to onset is more plausibly explained in terms of detection of existing disease following radiography for injury, particularly because of the predisposition to fracture, rather than in terms of a traumatic origin for Paget's disease. Deafness and arthropathy are known complications of Paget's disease, so the reporting of these disabilities as the most troublesome other chronic illness more often among index cases was to be expected and provides no new information on the aetiology of Paget's disease.

The excess of index cases in Occupation Unit Groups 24 and 25, compared with normal sibs, is difficult to interpret, but appears unlikely to reflect an occupational component in the pathogenesis of the disease. If anything, it might be expected that those involved in manual rather than non-manual work would be more likely to know of any Paget's disease present since physical stress is thought to encourage development of the disease (30). On the other hand, it is possible that some of the deficiency of cases involved in manual work could have been caused by the more severely affected changing to more sedentary jobs after the onset of disability. The overrepresentation of the higher social classes can be plausibly explained by better access to medical services (31) and therefore greater likelihood of diagnosis in the higher social classes, and by greater readiness of those in the higher social classes to become members of the National

Association for the Relief of Paget's Disease and to complete the questionnaire (32). The present data thus provide no convincing evidence that either occupation or social class contributes to the aetiology of Paget's disease.

Geographical location has been shown to have a marked influence on the prevalence of Paget's disease at the population level (1,5,6). However, it was not possible to demonstrate an association between geographical location and Paget's disease either within sibships (Table 4) or between sibships in the present sample. Any contribution to resemblance between relatives made by a common geographical location is therefore likely to be small, although, because of the large size of certain postcode areas, very localised clustering of concordant sibs could have occurred undetected.

Failure to demonstrate statistically significant cyclic variation in the distribution of years of birth for index cases could perhaps have been due to the small number of cases born in any particular year and to the fact that the 3-year periodicity was not present over all years studied. If future investigations are able to confirm the existence of cyclic variation in relative frequency of different years of birth for Paget's disease patients, this would be very suggestive of an infectious aetiology, with infection occurring at or around the time of birth. It is relevant to note that the majority of cases of serious infection by RSV are found in infants less than 12 months old, with the peak incidence at 1-2 months. This is in marked contrast to measles, against which a degree of protection during the first year of life is afforded by maternal antibody and where the age at infection is much more variable (33). A more pronounced year of birth effect would therefore be expected from RSV than from measles.

CONCLUSIONS

The familial nature of Paget's disease of bone has been confirmed. The aetiology of the disease is likely to be independent of other illnesses, occupation and social class. Similarity between relatives with respect to presence or absence of the disease is more readily explained by common genes than by common environment. Variation in age of onset of the disease may also have a genetic basis. The findings of the present study are consistent with the hypothesis that Paget's disease results from infection by a common and widespread virus, possibly at or around the time of birth, superimposed on genetic variation for susceptibility and perhaps severity of the disease. Further investigations of the possible year of birth effect might provide epidemiological support for the viral hypothesis.

Acknowledgements

The authors would like to thank the National Association for the Relief of Paget's Disease for financial support and access to their members, the patients themselves for their cooperation, Mrs Loraine Williamson for clerical assistance and Dr A.M. Davie and Mrs G.M. Raab for statistical advice in relation to cyclic variation. Dr. J. Davies of the Royal National Hospital for Rheumatic Diseases, Bath, and Dr Roger Smith of the Nuffield Orthopaedic Centre, Oxford, kindly contributed a small number of cases.

Table 1. Numbers of different types of relative who survived to at least 55 years of age and the numbers and percentages affected by Paget's disease. Numbers of index cases in brackets.

	Male Index (152)			Female Index (255)			Both Sexes (407)		
		Affected			Affected			Affected	
Type of relative	Total	No.	%	Total	No.	%	Total	No.	%
Fathers	113	1	0.88	172	11	6.40	285	12	4.21
Mothers	119	8	6.72	183	7	3.83	302	15	4.97
Brothers	148	1	0.68	257	7	2.72	405	8	1.98
Sisters	166	6	3.61	292	16	5.48	458	22	4.80
Sons	4	0	0.0	20	0	0.0	24	0	0.0
Daughters	12	0	0.0	23	0	0.0	35	0	0.0
Male Spouses	-	-	-	146	0	0.0	146	0	0.0
Female Spouses	110	0	0.0	-	-	-	110	0	0.0
Spouses' fathers	81	0	0.0	99	0	0.0	180	0	0.0
Spouses' mothers	94	2	2.13	103	0	0.0	197	2	1.02
Spouses' brothers	95	0	0.0	124	1	0.81	219	1	0.46
Spouses' sisters	107	0	0.0	120	0	0.0	227	0	0.0

Table 2. Mean age of onset for isolated and familial cases

	Number	Mean age of onset (years)	s.e.	Diff.	t	p
Isolated	327	56.98	0.72	3.49	2.24	0.02
Familial	95	53.49	1.38			

Table 3. Analysis of variance for age of onset, and comparison of within sibship variances for year of onset and age of onset.

	s.s.	d.f.	Mean square	F	p
Age of onset between families	11486	35	328.17	3.52	< 0.005
Age of onset within families	3720	40	93.12		
Year of onset within sibships	4759	29	164.29	2.20	< 0.025
Age of onset within sibships	2162	29	74.63		

Table 4. The distribution of sib pairs concordant and discordant for Paget's disease from 33 sibships by postcode area of birth and main postcode area of residence over three age ranges.

	Birth		0-20 yrs		21-40 yrs		> 40 yrs	
Postcode area	Same	Diff.	Same	Diff.	Same	Diff.	Same	Diff.
Concordant pairs	30	9	35	4	18	21	18	21
Discordant pairs	159	33	157	31	94	72	70	84
χ^2	0.41		0.54		1.01		0.01	
p	n.s.		n.s.		n.s.		n.s.	

Table 5. The numbers and proportions of cases of Paget's disease reported to have had a positive family history.

Source	Year	Number	Per cent
Gutman and Kasabach (21)	1936	4/115	3.5
Locke (22)	1943	10/48	20.8
Dickson et al (23)	1945	16/367	4.4
Rosenkranz et al (24)	1952	7/111	6.3
Galbraith (25)	1954	4/52	7.7
Galbraith et al (26)	1977	3/285	1.1
Present study		56/407	13.8

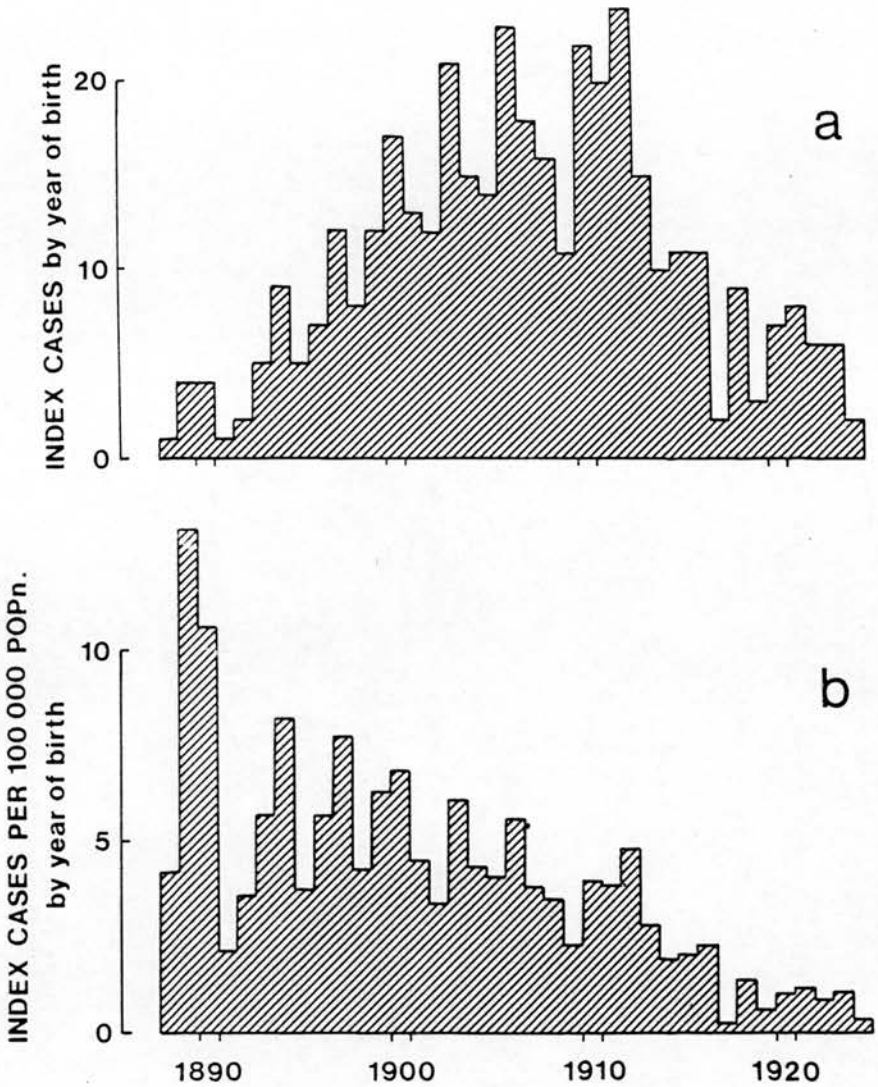


Figure 1. (a) Distribution of the 386 index cases born from 1888 to 1924, by year of birth. (b) The number of index cases born in England and Wales in each year from 1888 to 1924 (total 347) as a proportion of all persons with the same year of birth in the total population of England and Wales at 31st December 1980.

References

1. Barker D J P, Chamberlain A T, Guyer P B, Gardner M J. Paget's disease of bone: the Lancashire focus. Br Med J 1980; i: 1105-07.
2. Williams N J (editor). Diphosphonates and Paget's disease. Br J Clin Pract 1981; Symp Suppl 13.
3. McKusick V A. Mendelian inheritance in man. 5th ed. Baltimore: The Johns Hopkins University Press, 1978: 294-95.
4. Fotino M, Haymovits A, Falk C T. Evidence for linkage between HLA and Paget's disease. Transpl Proc 1977; 9: 1867-68.
5. Gardner M J, Guyer P B, Barker D J P. Radiological prevalence of Paget's disease of bone in British migrants to Australia. Br Med J 1978; i: 1655-57.
6. Guyer P B, Chamberlain A T. Paget's disease of bone in two American cities. Br Med J 1980; i: 985.
7. Singer F R. Paget's disease of bone: a slow virus infection? Calc Tiss Int 1980; 31: 185-87.
8. Rebel A, Basle K, Malkani K. Towards a viral aetiology for Paget's bone disease. Br J Clin Pract 1981; Symp Suppl 13: 9-14.
9. Mills B G, Singer F R, Weiner L P, Holst P A. Immunohistological demonstration of respiratory syncytial virus antigens in Paget's disease of bone. Proc Natl Acad Sci USA 1981; 78: 1209-13.
10. Mills B G, Stabile E, Holst P, Graham C. Antigens of two different viruses in Paget's disease of bone. J dent Res 1982; 61: 347.
11. Morgan-Capner P, Robinson P, Clewley G, Darby A, Pettingale K. Measles antibody in Paget's disease. Lancet 1981; i: 733.
12. Winfield J, Sutherland S. Measles antibody in Paget's disease. Lancet 1981; i: 891.

13. Singer F R, Mills B G, Weiner L P. Elevated serum paramyxovirus antibodies in patients with Paget's disease of bone. Clin Res 1978; 26: 533A.

14. Office of Population Censuses and Surveys. Classification of Occupations. London: HMSO, 1970.

15. World Health Organisation. International classification of diseases. Geneva: WHO, 1978.

16. Reid, I. Social class differences in Britain. London: Open Books, 1977.

17. Montague M F A. Paget's disease (osteitis deformans) and heredity. Am J Hum Genet 1949; 1: 94-95.

18. McKusick V A. Paget's disease of the bone. In Heritable disorders of connective tissue (3rd ed) St Louis: Mosby, 1972: 304-09.

19. Jones J V, Reed M F. Paget's disease: a family with six cases. Br Med J 1967; iv: 90-91.

20. Evens R G, Bartter F C. The hereditary aspects of Paget's disease (osteitis deformans). J Am Med Ass 1968; 206: 900-02.

21. Gutman A B, Kasabach H H. Paget's disease (osteitis deformans): analysis of 116 cases. Amer J Med Sci 1936; 191: 361-80.

22. Locke E A. quoted by Galbraith (26).

23. Dickson D D, Camp J D, Ghormley R K. Osteitis deformans: Paget's disease of the bone. Radiology 1945; 44: 449-70.

24. Rosenkrantz J A, Wolf J, Kaicher J J. Paget's disease (osteitis deformans); review of 111 cases. Archs Int Med 1952; 90: 610-33.

25. Galbraith H-J B. Familial Paget's disease of bone. Br Med J 1954; ii: 29.

26. Galbraith H-J B, Evans E C, Lacey J. Paget's disease of bone - a clinical and genetic study. Postgrad Med J 1977; 53: 33-39.

27. Smith R. Modern treatment of Paget's disease of bone. Prescribers' J 1982; 22: 23-31.

28. Office of Population Censuses and Surveys. The General Household Survey. Introductory Report. London: HMSO, 1973.

29. Falconer D S. The inheritance of liability to certain diseases, estimated from the incidence among relatives. Ann Hum Genet 1965; 29: 51-76.

30. Guyer P B. Bone stress as a factor in the pathogenesis of osteitis deformans. Br J Clin Pract 1981; Symp Suppl 13: 15-18.

31. Townsend P, Davidson N (eds). Inequalities in health. The Black report. Harmondsworth: Penguin Books, 1982.

32. Sheikh K, Mattingly S. Investigating non-response bias in mail surveys. J Epidemiol Community Health. 1981; 35: 293-96.

33. Fenner F, White D O. Medical virology. 2nd ed. New York: Academic Press, 1976: 386-405.

A DENTAL CONTRIBUTION TO MEDICAL GENETICS

The teeth and other oral tissues frequently show manifestations of more generalised disease, so that oral signs may be valuable in the diagnosis of certain systemic conditions, including inherited disorders. One such disorder is the X-linked form of hypohidrotic ectodermal dysplasia, carriers of which usually show only mild signs that often appear to be restricted to minimal hypodontia. However, some carriers can be identified from among female hypodontia cases in general by means of an abnormally low sweat pore count, so that the potential exists for a reasonably practical method of screening for carriers at the population level.

Hypothesis

Journal of Medical Genetics, 1981, **18**, 459-460

A dental approach to carrier screening in X linked hypohidrotic ectodermal dysplasia

SUMMARY The frequency of carriers of X linked hypohidrotic ectodermal dysplasia among females with hypodontia of the permanent dentition (excluding third molars) could be as high as 1 in 500, and among females with deciduous hypodontia could be as high as 1 in 50. Since it may be possible to identify carriers from among female hypodontia cases in general by virtue of a reduced sweat pore count, the potential exists for a reasonably practical method of screening for carriers at the population level.

The X linked form of hypohidrotic (anhidrotic) ectodermal dysplasia (XHED) is a severe disorder characterised by hypodontia, hypotrichosis, and hypohidrosis, in which affected males can succumb to brain damage and even death through hyperthermia. Expression in heterozygous females is variable but generally mild and the majority of carriers are likely to escape detection unless rigorous clinical criteria are employed by experienced observers. The counting of sweat pores has been used as a means of identifying carriers within families,[1-4] but is not a practical proposition on a much larger scale. On the other hand, the majority of schoolchildren undergo regular dental examinations and hypodontia, a lower than normal number of teeth caused by failure of development, is a simple objective criterion that could perhaps be of value in preliminary screening for carriers at the population level. The purpose of this short paper is simply to draw attention to the probable frequency of carriers of XHED among females with hypodontia.

Assuming equilibrium, normal fitness of carriers, and a mutation rate common to both sexes, the population incidence of carriers, c, can be estimated from the equation $c/m = (4 + 2f)/3$, where m and f are the incidence and fitness of affected males.[5] Substituting $f = 0{\cdot}6$ and a range for m of 1 in 100 000 to 1 in 10 000,[6 7] the population incidence of XHED carriers can be calculated as 1·73 to 17·3 per 100 000 females. Average figures for the prevalence of hypodontia among females from the general population are 6·0% for the permanent dentition (excluding third molars) but only 0·5% for the deciduous dentition,[8] that is, 6000 and 500 per 100 000, respectively. The prevalence of hypodontia among carriers of XHED, estimated from published reports of families, is 75% for the permanent dentition (excluding third molars) and 60% for the deciduous dentition.[9] Given the calculated incidence of XHED carriers, this provides ranges of 1·3 to 13·0 and 1·04 to 10·4 per 100 000 females for the population frequency of carriers with hypodontia of the permanent and deciduous dentitions, respectively. The table shows that using these figures the frequency of carriers among females with hypodontia of the permanent dentition is in the range $2{\cdot}17 \times 10^{-4}$ to $2{\cdot}17 \times 10^{-3}$, or approximately 1 in 5000 to 1 in 500, and the frequency of carriers among females with hypodontia of the deciduous dentition is in the range $2{\cdot}08 \times 10^{-3}$ to $2{\cdot}08 \times 10^{-2}$, or approximately 1 in 500 to 1 in 50. In a recent study,[9] sweat pore counts were markedly reduced in all of six XHED carriers but in none of eight control female hypodontia cases, the level of hypodontia in the permanent dentition (excluding third molars) ranging from one to seven missing teeth for the carriers and one to eight missing teeth for the otherwise apparently normal females. Sweat pore counts can therefore be used to distinguish between carriers of XHED with hypodontia and females whose teeth have failed to develop for other reasons.

Preliminary screening for hypodontia therefore brings population screening for carriers by sweat

Received for publication 21 January 1981

TABLE *The frequency of carriers of XHED among female hypodontia cases*

	Population frequency per 100 000 females			*Carrier frequency among female hypodontia cases*
	Carriers	*Carriers with hypodontia*	*Total hypodontia*	
Permanent dentition (excluding 3rd molars)	1·73–17·3	1·30–13·0	6000	$2{\cdot}17 \times 10^{-4} - 2{\cdot}17 \times 10^{-3}$
Deciduous dentition	1·73–17·3	1·04–10·4	500	$2{\cdot}08 \times 10^{-3} - 2{\cdot}08 \times 10^{-2}$

pore counting down to a reasonably practical level. However, the efficiency of such a screening method would depend not only on the proportion of carriers who have missing teeth, but also on the regularity with which carrier sweat pore counts fall below the normal range. The reported frequency of abnormally low sweat pore counts in small samples of carriers ranges from 20 to 25%[2 10] to 80 to 100%.[1 3 4 9] Furthermore, a reliable diagnosis of carrier status depends on the lack, or relative rarity, of alternative causes of hypohidrosis that could occur in conjunction with hypodontia, either as part of the same syndrome or simply by chance. Perhaps the most important possible drawback of the proposed screening method concerns the autosomal recessive form of hypohidrotic ectodermal dysplasia, since carriers of this disorder have a negligible chance of producing affected offspring. However, the available evidence suggests that carriers of the recessive form have normal dentitions, and normal sweat pore counts.[10 11] A summary of the clinical features (including sweat gland number and dental abnormalities) of several ectodermal dysplasia syndromes[12] indicates that none of these conditions, nor other disorders characterised by hypohidrosis,[13] is likely to jeopardise the potential of the proposed screening method.

Larger scale investigations than those already undertaken are required before the practicability and reliability of any such screening programme could be assessed, but it seems likely that a substantial proportion of carriers of XHED could be identified by sweat pore counting from among cases of hypodontia found at routine dental examinations.

The author is grateful to Professor A E H Emery for pointing out the problems involved in detecting carriers of XHED.

J A Sofaer

Department of Oral Medicine and Oral Pathology, University of Edinburgh, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR, and University Department of Human Genetics, Western General Hospital, Edinburgh EH4 2XU.

References

1 Frias JL, Smith DW. Diminished sweat pores in hypohidrotic ectodermal dysplasia: a new method for assessment. *J Pediatr* 1968;**72**:606–10.

2 Verbov J. Hypohidrotic (or anhidrotic) ectodermal dysplasia—an appraisal of diagnostic methods. *Br J Dermatol* 1970;**83**:341–8.

3 Settineri WMF, Salzano FM, De Melo e Freitas MJ. X-linked anhidrotic ectodermal dysplasia with some unusual features. *J Med Genet* 1976;**13**:212–6.

4 Pinheiro M, Freire-Maia N. Christ-Siemens-Touraine syndrome—a clinical and genetic analysis of a large Brazilian kindred. I. Affected females. *Am J Med Genet* 1979;**4**:113–22.

5 Holloway SM, Smith C. Equilibrium frequencies in X-linked recessive disease. *Am J Hum Genet* 1973;**25**: 388–96.

6 Stevenson AC, Kerr CB. On the distribution of frequencies of mutation to genes determining harmful traits in man. *Mutat Res* 1967;**4**:339–52.

7 Carter CO. Monogenic disorders. *J Med Genet* 1977;**14**: 316–20.

8 Brook AH. Dental anomalies of number, form and size: their prevalence in British schoolchildren. *J Int Ass Dent Child* 1974;**5**:37–53.

9 Sofaer JA. Hypodontia and sweat pore counts in detecting carriers of X-linked hypohidrotic ectodermal dysplasia. *Br Dent J* 1981 (in press).

10 Crump IA, Danks DM. Hypohidrotic ectodermal dysplasia. *J Pediatr* 1971;**78**:466–73.

11 Bartlett RC, Eversole LR, Adkins RS. Autosomal recessive hypohidrotic ectodermal dysplasia: dental manifestations. *Oral Surg* 1972;**33**:736–42.

12 Witkop CJ, Brearley LJ, Gentry WC. Hypoplastic enamel, onycholysis and hypohidrosis inherited as an autosomal dominant trait: a review of ectodermal dysplasia syndromes. *Oral Surg* 1975;**39**:71–86.

13 McKusick VA. *Mendelian inheritance in man.* 5th ed. Baltimore: Johns Hopkins University Press, 1978.

Requests for reprints to Dr J A Sofaer, Department of Oral Medicine and Oral Pathology, University of Edinburgh, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR.

NOVEMBER 17 1981

Hypodontia and Sweat Pore Counts in Detecting Carriers of X-linked Hypohidrotic Ectodermal Dysplasia

Brit. dent. J., 1981, 151, 327

J. A. SOFAER[1], PhD, BDS

The X-linked form of hypohidrotic ectodermal dysplasia is a severe inherited disorder in which affected males can succumb to brain damage and even death due to hyperthermia. For each son of a carrier of this disorder there is a 50 per cent chance of being affected, yet carriers themselves usually show only mild manifestations that frequently appear to be restricted to only minimal hypodontia. The present study indicates that at least some carriers can be identified from among hypodontia cases in general by virtue of a reduced sweat pore count. The frequency of carriers among females with hypodontia of the permanent dentition (excluding third molars) could be as high as 1 in 500, and among females with deciduous hypodontia could be as high as 1 in 50. The potential therefore exists for identifying women with a high risk of having a severely affected child from among patients with what is usually regarded as a rather unremarkable dental anomaly.

MALES affected by the X-linked form of hypohidrotic ectodermal dysplasia show the triad of hypodontia, hypotrichosis and hypohidrosis with a characteristic physiognomy. The skull tends to resemble an inverted triangle when viewed from in front, with frontal bossing and a depressed nasal bridge. The skin is soft, thin and dry, and the hair fine and usually blond. Eyelashes, and especially eyebrows, are often missing. The few teeth that may be present are sometimes retarded in eruption and frequently of a rudimentary conical shape. Other features include a susceptibility to asthma and eczema (Gorlin *et al.*, 1976).

The most dangerous aspect of the condition is the almost total failure of sweat gland development with the consequent inability to perspire. The result is that only mild exertion or infection can produce hyperthermia sufficient to cause permanent brain damage and even death. Nevertheless, the diagnosis of hypohidrotic ectodermal dysplasia is frequently overlooked in young children presenting with unexplained episodes of fever since, in infants, sparse hair is common and absence of teeth normal. Furthermore, the characteristic facies of the disorder is not fully developed until the second decade of life.

The Pattern of Inheritance

In families with X-linked hypohidrotic ectodermal dysplasia (XHED) it is not uncommon to find that early infant mortality has occurred among the male offspring, presumably because of hyperpyrexia associated with uncontrolled infection. These tragedies could be prevented, or at least reduced in number by identifying women at risk of having affected sons and giving appropriate genetic counselling.

The pattern of inheritance shown by XHED is typical of the majority of X-linked disorders. Males are most severely affected because when they have the abnormal allele (form of the gene) on their single X-chromosome there is no corresponding normal allele to moderate its effects. Females with the abnormal allele are nearly always heterozygous; that is, they have the abnormal allele on one member of their pair of X-chromosomes and the corresponding normal allele on the other. This is simply because the abnormal allele is rare and therefore unlikely to occur on both X-chromosomes in the same individual. Heterozygous females are known as 'carriers' of the disorder and show a generally rather mild, somewhat variable, degree of manifestation. The disorder is passed from a father, through his daughters, all of whom are carriers, to an average of half the sons of these carriers. Half the daughters of a carrier, on average, are also carriers. A consequence of this is that frequently the maternal uncles of an affected male are also affected. The condition is never passed directly from father to son. Occasionally affected males occur sporadically, without any known affected relatives. In these situations new mutations may have been responsible.

For each son of a carrier of XHED there is thus a 50 per cent chance of being affected, yet in these carriers manifestation of the disorder may be restricted, at least on superficial examination, to minimal hypodontia and/or peg shaped teeth. Carriers might therefore pass unnoticed among the body of hypodontia cases at large and consequently remain unaware of this genetic risk. The study reported here was therefore undertaken to establish whether it might be possible, by means of making sweat pore counts, to identify carriers of XHED from among female hypodontia cases.

The Prevalence of Hypodontia

The prevalence of hypodontia in the general population has been the subject of a number of studies summarised and supplemented by Brook (1974). The combined results of Brook's summary and original data show that hypodontia in the deciduous dentition is relatively rare, the frequency of affected individuals being in the region of 0·1 to 0·9 per cent among a total of approximately 20,000 children, with no evidence of a difference between the sexes. In the permanent dentition, however, hypodontia (excluding agenesis of the third molars) is much more

University of Edinburgh, Department of Oral Medicine and Oral Pathology, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR, and University Department of Human Genetics, Western General Hospital, Edinburgh EH4 2XU.

common, the proportion of those affected being in the range 3·5 to 6·5 per cent among a total of approximately 31,000 individuals. These studies also showed hypodontia in the permanent dentition to be consistently more frequent among females than males in the ratio of approximately 1·5:1. Based on these figures, the frequencies of individuals with hypodontia of the permanent dentition (excluding third molars) can therefore be taken as around 6 per cent for females and 4 per cent for males.

The prevalence of hypodontia in the permanent dentition of carriers of XHED can be estimated by combining data from reports of families in the literature. There are numerous such reports compatible with X-linked inheritance, with frequent mention of hypodontia and occasional mention of peg-shaped teeth among female relatives of affected males. However, reliable dental information on females for whom there is acceptable genetic (rather than clinical) evidence of their carrier status is limited. Table I lists a number of studies in which the dental status of carrier females is mentioned. The criterion for including a female in Table I was either having an affected father, or having an affected son and at least one other affected male relative (usually a brother, a nephew or another son). Having an affected son alone is not sufficient evidence of carrier status since this son could be the recipient of a new mutation. The prevalence of reported hypodontia of the permanent dentition (excluding third molars) among these carriers is 75 per cent, though it should be noted that the rigour of the dental diagnosis is likely to have been variable. Some of these females were reported to have no other signs of the disorder, while others had only mild signs that either might not have been detected by the untrained eye or could only be demonstrated by special tests for sweat gland number or function.

TABLE I.—THE PREVALENCE OF HYPODONTIA OF THE PERMANENT DENTITION, EXCLUDING THIRD MOLARS, AMONG CARRIERS OF XHED BY GENETIC CRITERIA

Authors	*Carriers studied*	*Carriers with hypodontia*
Glicklich and Rosenthal (1959)	2	2
Kerr *et al.* (1966)	4	3
Priest (1967)	2	1
Frias and Smith (1968)	3	0
Emery (1969)	8	6
Reed *et al.* (1970)	1	1
Verbov (1970)	2	2
Beahrs *et al.* (1971)	1	1
Crump and Danks (1971)	2	2
Familusi *et al.* (1975)	1	1
Settineri *et al.* (1976)	20	15
Pinheiro and Freire-Maia (1979)	5	4
Present study	2	2
Total	53	(75%) 40

The prevalence of hypodontia in the deciduous dentition of carriers is less easy to estimate, since either a good long-term dental history is required or the carriers must be in a restricted age range when examined. However, 3 of the 5 adult carriers (by genetic criteria) discussed by Pinheiro and Freire-Maia (1979) were reported to have had missing deciduous teeth. The level of deciduous hypodontia, relative to hypodontia of the permanent dentition, could therefore be much higher among carriers than among females from the general population.

Sweat Pore Counting

There is evidence to show that the counting of sweat pores per linear centimetre of epidermal ridge can distinguish between normal females and carriers of XHED (Frias and Smith, 1968; Verbov, 1970), although counts in carriers may not always fall outside the normal range (Crump and Danks, 1971). Sweat pores are the openings of the sweat gland ducts that are found along the crests of epidermal ridges. These pores can be counted either by direct inspection, or indirectly by using imprint or impression techniques. For the direct method the untreated skin of the subject, usually of the palms or fingertips, can be examined under a low-power stereomicroscope. Alternatively, the pores can be made more distinct by painting the skin with a 5 per cent solution of o-phthalaldehyde in xylene, shortly after which darkly stained puncta appear at the sweat gland orifices due to a reaction with ammonia. However, the dark pigment is retained in the skin for 2 or 3 days until the surface layers are shed (Juhlin and Shelley, 1967). Indirect methods, which have the advantage of providing a permanent record, have involved making cellulose acetate imprints or rubber base impressions of the skin, followed by pore counting under a low power stereomicroscope or from standardised photomicrograph enlargements. Acetate imprints can be made by painting a thin film of 2 per cent cellulose acetate and 0·35 per cent crystal violet in acetone onto the skin. When this is dry, two further layers of 2 per cent cellulose acetate alone are applied, and when these are dry the whole film is stripped off with cellulose adhesive tape and mounted, imprint side uppermost, on a microscope slide (Crump and Danks, 1971). For rubber base impressions (Verbov, 1970) the materials normally available in dental practice can be used.

Figure 1A shows the appearance of a fingertip impression from a 9-year-old child. The impressions of the sweat pores can be seen easily along the crests of clearly defined epidermal ridges. Figure 1B shows a fingertip

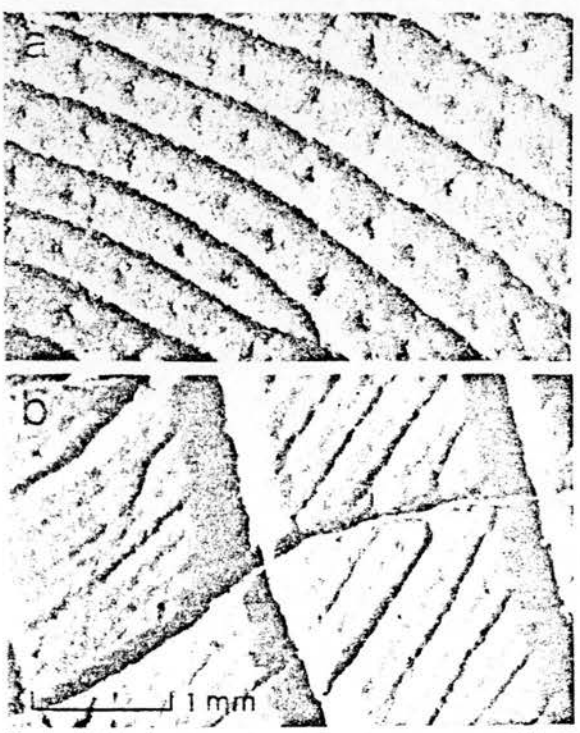


Fig. 1.—Rubber base impressions of fingertips of: A, a normal 9-year-old child and B, an affected 9-year-old boy.

impression from a 9-year-old boy with XHED. The ridges are relatively flat, making them less distinct, and in addition there are deeper criss-crossing furrows in the skin. There are no signs of any sweat pores. It is therefore not difficult to distinguish between this affected male and a normal individual.

Method

In order to investigate the possibility of using sweat pore counts to identify carriers of XHED from among female hypodontia cases, counts were made on fingertip impressions from 44 normal females ranging in age from 4 to 72 years, 2 known carriers, both daughters of the same affected male, and 4 probable carriers, all relatives of a second affected male from another family. These probable carriers were classified as such on the basis of their hypodontia or rather sparse hair. In addition, counts were made on 8 female hypodontia cases who were not suspected of being carriers of the disorder. The level of hypodontia in the permanent dentition (excluding third molars) ranged from 1 to 7 missing teeth for the carriers and 1 to 8 for the otherwise apparently normal hypodontia cases.

For each subject, impressions of the tips of the thumb and all 4 fingers of each hand were taken using Kerr's Permlastic Lightbodied Rubber Base. Mixed material was placed on the tip of each finger, and the subject asked to rest the fingertips gently on a sheet of paper until the material was set. When the fingers had been removed, the impressions remained attached to the paper without the need for adhesive. The impressions were examined and sweat pores counted under a stereomicroscope with a calibrated eyepiece graticule at a magnification of $\times 10$.

For each fingertip, 4 counts, each over 0·5 cm of ridge, were used to arrive at a representative count per centimetre. For each individual, the mean count with its standard error over all 10 figures was then plotted against age, as shown in figure 2. The results for normal subjects are consistent with those of Frias and Smith (1968) and

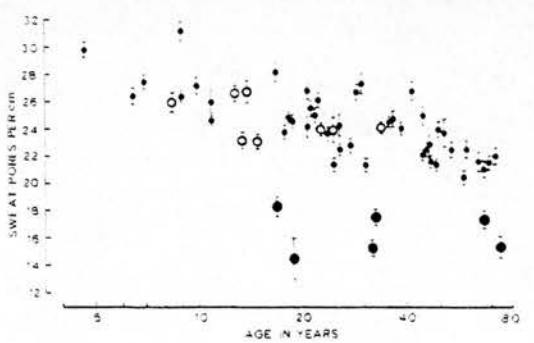


Fig. 2.—Mean sweat pore counts (± one standard error) for normal females (*small closed circles*), known and probable carriers (*large closed circles*) and otherwise apparently normal female hypodontia cases (*open circles*).

Crump and Danks (1971), showing a reduction from around 30 pores/cm at 4 years of age to around 22 pores/cm at ages over 70. Both known carriers and all 4 probable carriers showed an abnormally low number of sweat pores, and all 8 presumed otherwise normal hypodontia cases had counts in the normal range. On the basis of these results, it therefore appears to be quite possible to distinguish between female hypodontia cases who are, and those who are not, carriers of XHED.

Discussion

The Frequency of Carriers among Female Hypodontia Cases

It is difficult to estimate with accuracy the prevalence of the disorder in any given population unless effective screening procedures are adopted. In one survey where screening for XHED was carried out the frequency of affected males was found to be approximately 1 in 100,000 live male births (Stevenson and Kerr, 1967), but it is possible that in other populations the frequency of affected males could be as high as 1 in 10,000 (Carter, 1977). According to population genetic theory, and based on a relative reproductive fitness of 0·6 (60 per cent of normal) for XHED males (Stevenson and Kerr, 1967), the frequency of carriers should be approximately 1·73 times the frequency of affected males born into the population (Emery, 1976).

Frequencies of affected males in the range 1 in 100,000 to 1 in 10,000 therefore give corresponding carrier frequencies of approximately 1 in 60,000 to 1 in 6,000. Based on these carrier frequencies, and taking a figure for non-third-molar hypodontia of 6 per cent for females in general (Brook, 1974) and 75 per cent for carriers (Table I), the frequency of carriers among females with hypodontia of the permanent dentition (excluding third molars) is in the range of approximately 1 in 5,000 to 1 in 500. Because the relative prevalence of deciduous to permanent hypodontia is likely to be much higher among carriers than among females in general, the frequency of carriers is likely to be much higher among females with deciduous as opposed to permanent hypodontia. For example, taking the prevalence of deciduous hypodontia among females in general as 0·5 per cent (Brook, 1974) and among carriers as 60 per cent (3 out of 5 carriers by genetic criteria from Pinheiro and Freire-Maia, 1979), the frequency of carriers among females with hypodontia of the deciduous dentition is in the range of approximately 1 in 500 to 1 in 50.

It should, however, be borne in mind that the ability to identify carriers of XHED by the approach suggested here depends not only on their missing teeth but also on the regularity with which their sweat pore counts fall below the normal range. Furthermore, the diagnosis of carrier status using this approach depends on the lack, or relative rarity, of alternative causes of hypohidrosis that could occur in conjunction with hypodontia, either as part of the same syndrome or simply by chance.

Other Causes of Hypohidrosis

A relatively rare autosomal recessive form of hypohidrotic ectodermal dysplasia is known to exist (McKusick, 1978). For carriers of this form of the disorder there is a negligible risk of having an affected child, because affected children are almost invariably born into families in which both parents are carriers, and the likelihood of this kind of family occurring is very small. It is therefore important to be able to distinguish between carriers of the X-linked and autosomal recessive forms. Carriers of autosomal recessive disorders, if they are affected at all, usually show even milder signs than carriers of X-linked conditions. The available evidence suggests that this is the case for

hypohidrotic ectodermal dysplasia, since the parents of a family with the recessive form, who were presumably both carriers, had normal dentitions (Bartlett *et al.*, 1972). The parents of another recessive family had both normal dentitions and sweat pore counts (Crump and Danks, 1971).

Recessively inherited anhidrosis due to failure of sweat gland development has been reported as an isolated character in one family (Mahloudji and Livingston, 1967). Recessive anhidrosis also occurs in conjunction with congenital sensory neuropathy, but in this disorder the anhidrosis is primarily or exclusively neurogenic in origin since normal sweat glands have been demonstrated (McKusick, 1978). There appears to be no information on sweat pore counts in the carriers of these disorders, but both are evidently very rare.

Autosomal dominant conditions in which hypohidrosis has been found include anhidrotic ectodermal dysplasia with cleft lip and palate, and the amelo-onychohypohidrotic syndrome (McKusick, 1978). In the latter condition it is sweat gland function that is abnormal, with sweat pore counts for affected individuals in the normal range. Other signs include hypoplastic or hypocalcified enamel and nail dysplasia (Witkop *et al.*, 1975). Dominantly inherited hypohidrosis with hyperpigmentation and hyperkeratosis is also a recognised syndrome (Sparrow *et al.*, 1976). However, in these dominant conditions the question of mild hypohidrosis alone does not arise, all individuals with the abnormal gene usually showing the complete syndrome.

In Fabry disease (diffuse angiokeratoma), an X-linked disorder, sparse or atrophic sweat glands are found in conjunction with clusters of dark red keratotic angiectases of the skin, corneal opacities and defective renal function (Gorlin *et al.*, 1976). Carrier females can frequently be identified because of corneal involvement (McKusick, 1978). In the amelocerebrohypohidrotic syndrome, for which either X-linked or autosomal recessive inheritance is possible on present evidence, hypohidrosis associated with decreased numbers of sweat glands occurs with severe seizures, progressive mental retardation and hypoplasia of the enamel (Witkop and Sauk, 1976).

The relationship between sweat gland number and function and abnormalities of the dentition and other ectodermally-derived structures has been summarised for several ectodermal dysplasia syndromes by Witkop *et al.* (1975).

Conclusion

For each son of a carrier of XHED there is a 50 per cent chance of being affected. Dental practitioners could have an important role to play in detecting carriers since 75 per cent of them show some degree of hypodontia of the permanent dentition (excluding third molars). Sweat pore counting is able to identify at least some carriers of XHED from among females with hypodontia of the permanent teeth. However, any programme for detecting carriers based on hypodontia and sweat pore counting is likely to produce a much better return if only females with deciduous hypodontia are included. Larger scale investigations are required before the practicability and reliability of any such screening programme could be assessed, but it is reasonable to say at this stage that the potential exists for identifying women with a high risk of having a severely affected child from among patients with what is usually regarded as a rather unremarkable dental anomaly.

ACKNOWLEDGMENTS

The author is grateful to Professor A. E. H. Emery for pointing out the problems involved in detecting carriers of XHED, and also for an introduction to a family with the disorder. The second family was found through Edinburgh Dental Hospital records.

REFERENCES

Bartlett, R. C., Eversole, L. R., and Adkins, R. S. (1972) Autosomal recessive hypohidrotic ectodermal dysplasia: dental manifestations. *Oral Surg.*, **33**, 736–742.

Beahrs, J. O., Lillington, G. A., Rosan, R. C., Russin, L., Lindgren, J. A., and Rowley, P. T. (1971) Anhidrotic ectodermal dysplasia: predisposition to bronchial disease. *Ann. Int. Med.*, **74**, 92–96.

Brook, A. H. (1974) Dental anomalies of number form and size: their prevalence in British school children. *J. Int. Ass. Dent. Child.*, **5**, 37–53.

Carter, C. O. (1977) Monogenic disorders. *J. med. Genet.*, **14**, 316–320.

Crump, I. A., and Danks, D. M. (1971) Hypohidrotic ectodermal dysplasia. *J. Pediat.*, **78**, 466–473.

Emery, A. E. H. (1969) *In* Selected Topics on Genital Anomalies and Related Subjects, p. 812. Edits. M. N. Rashad and W. R. M. Morton. Charles C. Thomas, Springfield, Ill.

—— (1976) Methodology in Medical Genetics, pp. 30–31. Churchill Livingstone, Edinburgh.

Familusi, J. B., Jaiyesimi, F., Ojo, C. O., and Ed. B. Attah (1975) Hereditary anhidrotic ectodermal dysplasia: studies in a Nigerian family. *Arch. Dis. Child.*, **50**, 642–647.

Frias, J. L., and Smith, D. W. (1968) Diminished sweat pores in hypohidrotic ectodermal dysplasia: a new method for assessment. *J. Pediat.*, **72**, 606–610.

Glicklich, L. B., and Rosenthal, I. M. (1959) Anhidrotic ectodermal dysplasia: use of silver nitrate plate to detect anhidrosis. *J. Pediat.*, **54**, 19–26.

Gorlin, R. J., Pindborg, J. J., and Cohen, M. M. (1976) Syndromes of the Head and Neck. McGraw-Hill, New York.

Juhlin, L., and Shelley, W. B. (1967) A stain for sweat pores. *Nature (Lond.)*, **312**, 408.

Kerr, C. B., Wells, R. S., and Cooper, K. E. (1966) Gene effect in carriers of anhidrotic ectodermal dysplasia. *J. med. Genet.*, **3**, 169–176.

McKusick, V. A. (1978) Mendelian Inheritance in Man, 5th ed. The Johns Hopkins University Press, Baltimore.

Mahloudji, M., and Livingston, K. E. (1967) Familial and congenital simple anhidrosis. *Amer. J. Dis. Child.*, **113**, 477–479.

Pinheiro, M., and Freire-Maia, N. (1979) Christ-Siemens-Touraine syndrome—a clinical and genetic analysis of a large Brazilian kindred: I. Affected females. *Amer. J. med. Genet.*, **4**, 113–122.

Priest, J. (1967) Dermatoglyphics in ectodermal dysplasia. *Lancet*, **2**, 1093.

Reed, W. B., Lopez, D. A., and Landing, B. (1970) Clinical spectrum of anhidrotic ectodermal dysplasia. *Arch. Dermatol.*, **102**, 134–143.

Settineri, W. M. F., Salzano, F. M., and De Melo e Freitas, M. J. (1976) X-linked anhidrotic ectodermal dysplasia with some unusual features. *J. med. Genet.*, **13**, 212–216.

Sparrow, G. P., Samman, P. D., and Wells, R. S. (1976) Hyperpigmentation and hypohidrosis. (The Naegeli-Franceschetti-Jadassohn syndrome): report of a family and review of the literature. *Clin. exp. Derm.*, **1**, 127–140.

Stevenson, A. C., and Kerr, C. B. (1967) On the distributions of frequencies of mutation to genes determining harmful traits in man. *Mutation Res.*, **4**, 339–352.

Verbov, J. (1970) Hypohidrotic (or anhidrotic) ectodermal dysplasia—an appraisal of diagnostic methods. *Brit. J. Derm.*, **83**, 341–348.

Witkop, C. J., jun., Brearley, L. J., and Gentry, W. C., jun. (1975) Hypoplastic enamel, onycholysis and hypohidrosis inherited as an autosomal dominant trait: a review of ectodermal dysplasia syndromes. *Oral Surg.*, **39**, 71–86.

——, **and Sauk, J. J., jun.** (1976) Heritable defects of enamel. *In* Oral Facial Genetics. Edits. R. E. Stewart and G. H. Prescott, Mosby, St Louis.

MISCELLANEOUS

Studies of the genes 'tabby' and 'crinkled' in the mouse (papers 1-3) led to the first two papers in this section. In one, different alleles of 'tabby' are shown to have different dominance relationships, and in the other, the non-allelic genes 'tabby' and 'crinkled' are found to have additive effects on tooth size. The third paper is of completely non-dental interest, being concerned with the possibility that single genes may be responsible for unusually high IQ scores, and the fourth arose out of the family study of Paget's disease (paper 23), being an investigation of dental extraction history in Paget's disease patients and their normal spouses.

DOMINANCE IN THRESHOLD CHARACTERS. A COMPARISON OF TWO TABBY ALLELES IN THE MOUSE

J. A. SOFAER AND C. J. MACLEAN

Human Genetics Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014

Received August 5, 1969

TWO alleles of the sex-linked gene tabby, *Ta* and *Ta*^{c}, reduce the number of secondary facial vibrissae in the mouse. Dominance on the phenotypic level in this system is considerably affected by background modification, so that estimates of dominance based directly on observations are difficult to interpret. The present analysis was based on a model which allowed transformation of the discontinuous observations to a postulated continuous scale of amount of gene product. With this accomplished, the dominance relationships of the alleles were investigated at a more fundamental level, relatively independent of genetic background.

MATERIALS AND METHODS

The original tabby allele (*Ta*), here called *Ta*^{f}, arose in a strain selected for large size on a low plane of nutrition (FALCONER 1953). *Ta*^{c} arose in a line selected for body weight (Mouse News Letter 1966), and resembles another tabby allele of independent origin, *Ta*^{j} (Mouse News Letter 1963). The effect of *Ta*^{f} and *Ta*^{c} on vibrissa number was examined for each allele on its original stock background, and on the backgrounds produced after a number of crosses to the inbred strains, JU/Fa and A/Fa. These strains differed in their ability to favor mutant expression (SOFAER 1969).

For each allele, the material collected was composed of five groups of animals according to background genotype: the stock background; the background after one cross to JU (½ JU), and after two crosses to JU (¾ JU), and the background after one cross to A (½ A), and after two crosses to A (¾ A). The *Ta*^{f} (¾ JU) group could not be completed because of poor fertility and was therefore not examined. All animals were the progeny of heterozygous mothers and tabby fathers, and all four genotypes of offspring were scored (*Ta*+ and *TaTa* ♀♀, and + and *Ta* ♂♂). The number of individuals of each genotype in each background varied between 21 and 26.

FRASER, NAY and KINDRED (1959) showed that there was no significant difference between tabby homozygotes and tabby hemizygotes with respect to vibrissa number. These two genotypes were therefore pooled so that in each background group for each allele there were three major-genotype classes of animals: +, *Ta*+, and *TaTa* plus *Ta*. Only the mean vibrissa scores of each of these genotypic classes were available for the analysis.

The secondary facial vibrissae are divided into four groups. On each side of the face there are 1 or 2 supraorbital, 0 or 1 postorbital, and 0, 1, or 2 postoral vibrissae; and beneath the chin there is a single group of 1, 2, or 3 interramal vibrissae. Animals with scores outside these ranges are rare, and none occurred in the present study. These four groups appear to be under nearly independent genetic control (FRASER, NAY and KINDRED 1959; FRASER and KINDRED 1962; SOFAER 1969). Any analysis of vibrissa number is then best carried out for each group separately.

THE MODEL

The model on which the analysis was based assumed a continuous scale of gene

product expressed on the phenotypic level in discrete categories (each phenotypic category being a particular number of vibrissae in a given vibrissa group). The values on the gene product scale corresponding to the different phenotypic categories were taken to be separated by constant thresholds. Within each background, each major-genotype class of animals was assumed to be normally distributed on the gene-product scale and, since animals of the same class were always to some extent inbred, the bulk of the variance within each class was considered to be environmental rather than genetic. As only mean vibrissa scores were available, the variance within all classes was of necessity assumed to be the same.

For the three bilateral vibrissa groups, the scores on the two sides of one individual, say L_i and R_i, can be regarded as two independent realizations of the same vibrissa-producing potential. Thus for these groups, the number of phenotypic categories was taken as the number of possible phenotypes on a single side (1 or 2 vibrissae at the supraorbital site, 0 or 1 at the postorbital site, and 0, 1 or 2 at the postoral site). The mean score of a class of N animals was then $[\Sigma(L_i + R_i)]/2N$. For the interramal group there were three possible phenotypes at this single site (1, 2 or 3 vibrissae), and the mean of a class of N animals was simply taken as $(\Sigma X_i)/N$, where X_i was an individual's interramal score. Thus a two-phenotype category or one-threshold model was used for the supraorbital and postorbital groups, and a three-phenotype category or two-threshold model was used for the postoral and interramal groups.

The mean phenotype of a class of animals clearly depends on how the distribution of the class on the gene product scale is partitioned by the thresholds. Figure 1 illustrates the two-threshold situation. The one-threshold situation can be re-

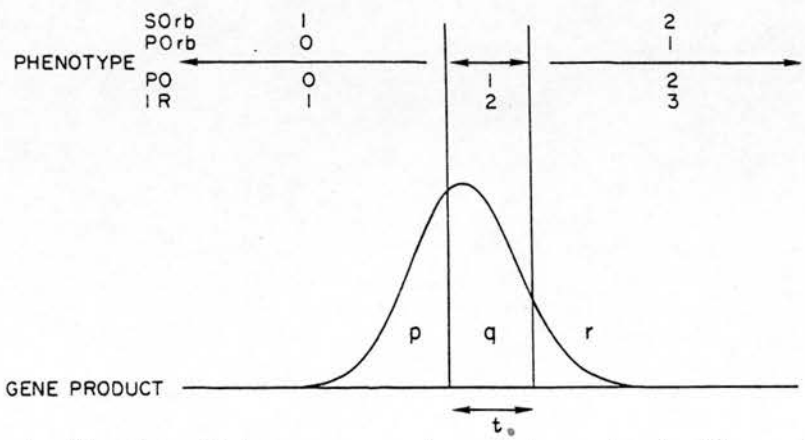


FIGURE 1.—The relationship between gene product and phenotypic scales. The two thresholds, separated by t standard deviations, divide the distribution of a class of individuals on the gene-product scale into those with minimum, intermediate, and maximum vibrissa scores, in the proportions p, q, and r, respectively. When $t = 0$, then $q = 0$, and the distribution is divided by a single threshold in the proportions p and r. For the purposes of illustration, the supraorbital (SOrb), postorbital (POrb), postoral (PO), and interramal (IR) phenotypic scales have been superimposed. This is not meant to imply that they are equivalent.

garded as a special case with the threshold interval, t, being equal to zero. If the scores given to each phenotypic category when there are two thresholds are n, $n+1$, $n+2$, and if p, q, and r are the proportions of a distribution that fall, respectively, below both thresholds, between the two thresholds, and above both thresholds, then the mean phenotypic score produced by that distribution is: $np + (n+1)q + (n+2)r$. If when $t = 0$ the scores given to each phenotypic category are n and $n+1$, and if p and r are the proportions of the distribution that then fall. below and above, respectively, the single threshold, then the mean phenotypic score is: $np + (n+1)r$.

Consider a series of distributions of given variance spread out along the gene-product scale. The means of these distributions plotted against their corresponding mean phenotypes produce a sigmoid curve. The shape of this curve depends on the relationship between the common standard deviation of the distributions (σ) and the threshold interval (t). Different values of t in relation to σ produce different curves. When $t = 0$, and there is only one threshold, the resulting curve is simply that of the cumulative normal distribution. As t increases, a central plateau in the curve appears. The size of this plateau is an indication of the range in which changes on the gene-product scale have little effect on moving the mean phenotype away from the intermediate value. It is therefore an expression of canalization at this level. Curves for $t = 0$, σ, 2σ and 3σ are illustrated in Figure 2.

These curves, used inversely, represent possible transformations of the data

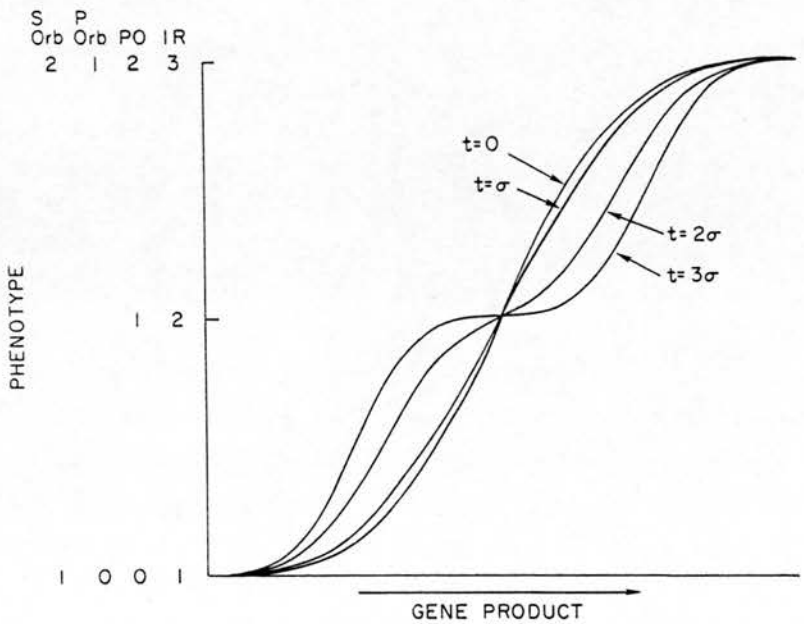


FIGURE 2.—The relationship between gene product and phenotypic means produced by a series of normal distributions of given variance spread out along the gene-product scale across two thresholds separated by t standard deviations. All curves have been adjusted to the same range. SOrb, POrb, PO and IR refer to supraorbital, postorbital, postoral and interramal vibrissa number.

from the observed mean vibrissa scores to their corresponding class-distribution means on the gene-product scale. The choice of an appropriate transformation allows dominance to be investigated at this more fundamental level.

The appropriate transformation for the one-threshold situation is clearly represented by the curve of $t = 0$. The choice of transformation for the two-threshold situation is based on the postulate that differences between means of different classes on the gene-product scale are due not only to allele substitution at the tabby locus but also to differences in genetic background, and that a given background change moves all class means on the gene-product scale by the same amount in the same direction. Thus, over a number of backgrounds, the variances due to background change at each of the three major-genotype levels are assumed to be equal on the gene-product scale. The transformation which minimizes the differences between these three backround variances is therefore considered the most appropriate. For practical purposes, this is represented by the curve which produces, in the transformed data, a ratio between a combined background variance for its extremes (mutant and wild-type homozygote and hemizygote levels), say V_x, and a background variance for its middle (heterozygote level), say V_m, which is closest to unity.

RESULT AND DISCUSSION

The observed mean vibrissa scores for each major-genotype class in each background are shown in Table 1.

In the supraorbital and postorbital groups there were no animals with less than the maximum number of vibrissae in all the wild-type classes. The positions of the distribution means of these classes on the gene-product scale could, therefore, not be estimated. Dominance calculations for these two groups were consequently restricted to untransformed data.

TABLE 1

Mean vibrissa scores for the three major-genotype classes (+, Ta+, TaTa *and* Ta) *in different genetic backgrounds at the supraorbital (SOrb), postorbital (POrb), postoral (PO), and interramal (IR) sites*

Allele and background	SOrb			POrb			PO			IR		
	+	*Ta*+	*TaTa* plus *Ta*	+	*Ta*+	*TaTa* plus *Ta*	+	*Ta*+	*TaTa* plus *Ta*	+	*Ta*+	*TaTa* plus *Ta*
Ta^f stock	4.00	3.86	2.12	2.00	1.68	0.00	3.59	2.00	1.82	2.86	2.05	1.21
Ta^f ½*JU*	4.00	3.68	2.00	2.00	1.82	0.00	3.59	1.95	0.91	2.95	2.41	1.28
Ta^f ½*A*	4.00	3.50	2.24	2.00	1.55	0.00	3.95	2.00	1.45	2.86	1.55	1.05
Ta^f ¾*A*	4.00	3.45	2.03	2.00	1.27	0.00	3.86	2.00	0.98	2.50	1.14	1.05
Ta^c stock	4.00	3.81	2.10	2.00	2.00	0.05	3.95	2.67	1.65	2.41	2.33	1.26
Ta^c ½*JU*	4.00	4.00	2.04	2.00	1.96	0.00	3.32	2.00	0.52	2.91	2.74	1.37
Ta^c ¾*JU*	4.00	3.77	2.03	2.00	1.36	0.00	2.86	1.86	0.14	2.68	2.27	1.03
Ta^c ½*A*	4.00	3.95	2.34	2.00	1.77	0.00	3.91	2.18	1.45	2.77	1.77	1.03
Ta^c ¾*A*	4.00	3.59	2.27	2.00	1.73	0.03	3.95	2.09	1.41	2.55	1.64	1.03

Each row contains data collected for either Ta^f or Ta^c in one background group. Each figure represents the mean score at one site in one class of animals. Figures in the bilateral groups are the means of individuals, not sides.

The postoral and interramal observations were transformed for several different values of t, and estimates of V_x and V_m were made on each transformed scale. The two background variances were estimated by first calculating the squared deviations of the mean vibrissa scores of each genotypic class for each allele, from its own within-genotype within-allele mean. The deviations of the wild-type and tabby homozygote or tabby hemizygote classes were then averaged to arrive at an estimate of V_x, and the deviations of the heterozygote classes were averaged to arrive at an estimate of V_m.

In the postoral group, the two variances were equal with a t value between 2σ and 3σ. The value of $t = 2.5\sigma$, with $V_x/V_m = 0.94$, was taken to be representative of this. At $t = 2\sigma$, $V_x/V_m = 2.06$; and at $t = 3\sigma$, $V_x/V_m = 0.49$. The transformations included in this range are enclosed in the area between the $t = 2\sigma$ and $t = 3\sigma$ curves in Figure 2.

In the interramal group, the two variances were most nearly equal when $t = 0$, with $V_x/V_m = 0.53$. This clearly could not have been the true value of t as some mice did have 2 interramal vibrissae. However, transformation is rather insensitive to changes in t in the range $t = 0$ to $t = \sigma$. This is illustrated in Figure 2 by the similarity of the $t = 0$ and $t = \sigma$ curves. A small threshold interval would therefore be just as compatible with this result as none at all. At $t = \sigma$, V_x/V_m was only reduced to 0.34.

The degree of dominance is defined as d/a where $2a$ is the difference between wild-type and mutant homozygote (or hemizygote) values, and where d is the amount by which the heterozygote exceeds $\overline{H}$, the mid-homozygote point. Thus if $d = 0$, then $d/a = 0$, and there is no dominance; if $d = +a$, then $d/a = +1$, and the wild-type allele is completely dominant over the mutant allele; and if $d = -a$, then $d/a = -1$, and the mutant allele is completely dominant over the wild-type allele.

The degree of dominance on the gene-product scale was calculated for each allele in each genetic background following transformation with $t = 2\sigma$, 2.5σ, and 3σ, for the postoral group; and transformation with $t = 0$ and $t = \sigma$ for the interramal group. The results, together with those of similar calculations in the untransformed data of all four groups, are illustrated in Figure 3. Each point in Figure 3 represents the degree of dominance calculated from the means of the three major-genotype classes for one allele in one background group.

The tendency at the phenotypic level was for a greater degree of dominance over wild-type to be associated with Ta^l than with Ta^c, but there was considerable overlap between the alleles in all vibrissa groups. Transformation of the postoral and interramal data had a marked effect on this overlap. All transformations in the ranges $t = 2\sigma$ to $t = 3\sigma$ for the postoral group, and $t = 0$ to $t = \sigma$ for the interramal group, reduced overlap. The transformations estimated to be the most appropriate on the basis of equality of background variance ($t = 2.5\sigma$ for the postoral group, and $t = 0$ for the interramal group) produced complete and maximal separation between alleles, and resulted in minimal variance of degree of dominance within alleles. The clarity of these results suggests that the assumptions involved in the model were not unreasonable.

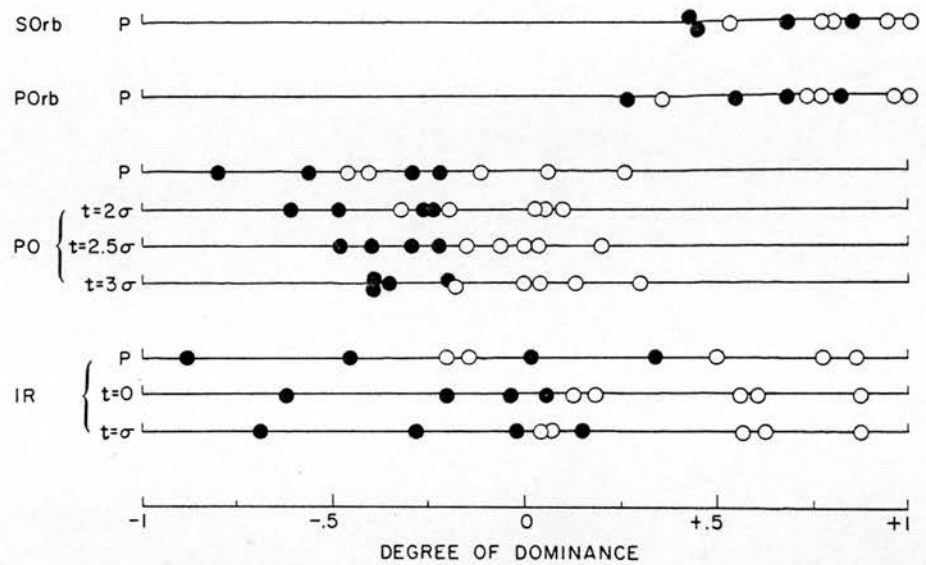


FIGURE 3.—The degree of dominance on the phenotypic scale (P) for the supraorbital (SOrb), postorbital (POrb), postoral (PO), and interramal (IR) vibrissa groups; and the degree of dominance on transformed scales for different values of t in the postoral and interramal groups. Each point represents the degree of dominance associated with one allele in one background genotype group. Closed circle = Ta^l; open circle = Ta^c.

The nature of the dominance calculation is such that inequality of background variance at the three major-genotype levels imposes an association between d/a and the general level of expression in any one background, which can be represented by $\overline{H}$, the mid-homozygote point. If the extreme genotypes vary more than the heterozygote, d/a will be negatively correlated with $\overline{H}$. This was the case with the postoral vibrissa scores, where the correlation between d/a and $\overline{H}$ was $r = -.80$. Conversely, if the extreme genotypes vary less than the heterozygote, d/a will be positively correlated with $\overline{H}$. This was the case with the interramal scores, where the correlation of d/a with $\overline{H}$ was $r = +.42$. An indication of the efficiency of the most appropriate transformations is that, in the transformed data, the correlation of d/a with $\overline{H}$ demonstrated no significant association between dominance and general level of expression ($r = -.14$ and $r = +.15$ in the postoral and interramal groups, respectively).

These transformations increased the correlation between degree of dominance in the postoral and interramal groups from $r = .59$ (untransformed) to $r = .73$ (transformed). This increase with transformation is good evidence for a real dominance association between these two groups. The correlation between degree of dominance in the untransformed supraorbital and postorbital data was also $r = .59$, but, as no transformation could be made, it is possible that $r = .59$ represents an association between the general levels of expression in the two groups. However, as evidence that it represents a dominance association, at least

TABLE 2

Values of d/a *on transformed scales for* Ta^f *and* Ta^c, *and D, the absolute value of the difference between them in different backgrounds, for the postoral (PO) and interramal (IR) vibrissa groups*

		¾ JU	½ JU	Stock	½ A	¾ A
PO ($t=2.5\sigma$)	Ta^f	..	—.29	—.48	—.40	—.22
	Ta^c	—.06	+.04	+.20	.00	—.15
	D	..	0.33	0.68	0.40	0.07
IR ($t=0$)	Ta^f	..	+.06	—.03	—.20	—.62
	Ta^c	+.60	+.56	+.87	+.12	+.18
	D	..	0.50	0.90	0.32	0.80

to some extent, the correlations of d/a values of each of these two groups with d/a on the transformed scale of the postoral and interramal groups (shown above to be independent of general level of expression) were all around r = .50. Thus, common fluctuations in the degree of dominance, for whatever reason, appear to be expressed in all four vibrissa groups.

Finally, it should be mentioned that this demonstration of a dominance difference does not necessarily imply an intrinsic difference between the alleles. There is evidence that gene activity on the X chromosome is controlled from an "inactivation center," and that different X chromosomes may have different inactivation properties (RUSSELL 1964; CATTANACH and ISAACSON 1967). Moreover, the inactivation center appears to be in the neighborhood of the tabby locus (RUSSELL and MONTGOMERY 1965). Thus, an apparent difference between alleles could be accounted for by a difference between closely linked controlling factors derived from the two mutant stocks. Evidence for the existence of controlling factors is provided by the fact that the difference between the alleles appeared to be reduced by crossing. Table 2 shows d/a values for Ta^f and Ta^c, and the absolute value of the difference between them (D), for the different levels of inbred backgrounds on the best transformed scales of the postoral and interramal vibrissa groups. The tendency was for D to decrease as the background was made more common to both alleles.

The authors are grateful to Professor D. S. FALCONER for suggesting the possible existence of a dominance difference between the alleles, and to Professor FALCONER, the staff of the Human Genetics Branch, NIDR, and one of the two reviewers of the manuscript for helpful criticism. The material was collected by the first author at the Institute of Animal Genetics, Edinburgh, Scotland.

SUMMARY

In all the secondary facial groups of vibrissae there was a suggestion of a difference in dominance between Ta^f and Ta^c on a number of genetic backgrounds at the phenotypic level. Common fluctuations in dominance appeared to be reflected

in all vibrissa groups. Observed postoral and interramal vibrissa scores were each transformed to a postulated continuous scale of amount of gene product, and in each case the transformation judged to be the most appropriate on the basis of other criteria resulted in complete separation of the alleles with respect to degree of dominance, minimal variance of degree of dominance within alleles, and a high positive correlation between degrees of dominance in these two vibrissa groups. There is therefore little doubt that there was a real dominance difference associated with the alleles at a fundamental level. However, at least some of this difference appeared to be attributable to a difference between controlling factors derived from the two original mutant stocks.

LITERATURE CITED

CATTANACH, B. M. and J. H. ISAACSON, 1967 Controlling elements in the mouse X chromosome. Genetics **57**: 331–346.

FALCONER, D. S., 1953 Total sex-linkage in the house mouse. Z. Vererbl. **85**: 210–219.

FRASER, A. S. and B. M. KINDRED, 1962 Selection for an invariant character, vibrissa number, in the house mouse. III. Correlated responses. Australian J. Biol. Sci. **15**: 188–206.

FRASER, A. S., T. NAY and B. M. KINDRED, 1959 Variation of vibrissa number in the house mouse. Australian J. Biol. Sci. **12**: 331–339.

Mouse News Letter, 1963. **29**: 40.

Mouse News Letter, 1966. **35**: 24.

RUSSELL, L. B., 1964 Another look at the single-active X hypothesis. Trans. N.Y. Acad. Sci. **26**: 726–736.

RUSSELL, L. B. and C. S. MONTGOMERY, 1965 The use of X–autosome translocations in locating the X chromosome inactivation center. Genetics **52**: 470–471.

SOFAER, J. A., 1969 Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. J. Embryol. Exptl. Morphol. **22**: 207–227.

Genet. Res., Camb. (1979), **33**, *pp.* 169–174
With 1 text-figure
Printed in Great Britain

Additive effects of the genes *tabby* and *crinkled* on tooth size in the mouse

By J. A. SOFAER

University of Edinburgh, Department of Oral Medicine and Oral Pathology, Edinburgh EH1 1NR and
Department of Human Genetics, Western General Hospital, Edinburgh EH4 2XU

(*Received* 19 *January* 1979)

SUMMARY

The semi-dominant *X*-linked gene tabby (*Ta*) in the mouse and its recessive autosomal mimic crinkled (*cr*) produce the same mutant syndrome involving abnormalities of the hair, teeth and certain exocrine glands. Previous work has provided some indication of interaction between these loci in terms of vibrissa number. The results of the present study demonstrate that, for tooth size, mice doubly heterozygous for tabby and crinkled show a more extreme phenotype than either heterozygotes for tabby or crinkled alone.

1. INTRODUCTION

The semi-dominant *X*-linked gene tabby (*Ta*) in the mouse, its two recessive autosomal mimics crinkled (*cr*) and downless (*dl*), and its one dominant autosomal mimic sleek (*Slk*), appear to produce the same mutant syndrome involving abnormalities of the hair, teeth and certain exocrine glands (Falconer, Fraser & King, 1951; Falconer, 1953; Grüneberg, 1965, 1966*a*, *b*, 1971; Cattanach, 1975; Sofaer, 1969*a*, *b*, 1977). Crinkled is located on chromosome 13, and both downless and sleek on chromosome 10. Linkage tests have shown *dl* and *Slk* to be less than 4·8 map units apart with 95% certainty, though allelism has not been demonstrated (Crocker, 1978).

The most general effect of the mutant genes on the dentition is to reduce tooth size, but, at least for the upper and lower first molars of tabby heterozygotes, there are three fairly distinct levels of reduction. The most severely reduced first molars are always found adjacent to abnormal additional teeth. On the other hand, minimally reduced and moderately reduced first molars do not occur with supernumerary teeth, though it seems likely that each moderately reduced first molar was associated with an unsuccessful attempt to form a supernumerary tooth during its early development (Sofaer, 1975).

Previous work has provided some indication of interaction between *Ta* and *cr* in terms of vibrissa number. However, the results were difficult to interpret in that there were instances of both enhancement and reduction of the normal mutant effect in doubly mutant animals (Kindred, 1967). The present report describes a study of molar tooth size in which mice doubly heterozygous for tabby and

0016-6723/79/2828-7370 $01.00

crinkled were compared with tabby heterozygotes and crinkled heterozygotes, the object being to provide further evidence of interaction between these two loci.

2. MATERIAL AND METHOD

The tabby allele used was that originally designated Ta^c (Roberts, 1966). This can now be regarded as equivalent to the allele Ta^j of earlier and independent origin (Stevens, 1963). Tabby and crinkled were transferred to a largely common genetic background by crossing to the inbred strain *JU/Fa*. Four groups of mice were examined: *JU/Fa* animals, wild type for *Ta* and *cr*, and *Ta/+*, *+/cr* and *Ta/+ . +/cr* mice with backgrounds produced by either two or three crosses to *JU/Fa*. Three of the groups were composed of around 25 animals each, but the fourth, the doubly heterozygous group, consisted of only nine animals because of poor fertility. In contrast to the *Ta/+* and *Ta/+ . +/cr* groups, the inbred and *+/cr* groups contained males and females in approximately equal numbers. The *Ta/+* and *Ta/+ . +/cr* animals were also all the progeny of *Ta/Ta* mothers, whereas mice of the *+/cr* group were the progeny of heterozygous mothers. However, these differences are unlikely to confound the results since previous work has estimated the proportion of the total variance of tooth size due to the difference between sexes at only about 1% (Bader, 1965), and no evidence of a difference in maternal effect on the mutant phenotype has been found between *Ta/+* and *Ta/Ta* mothers (Kindred, 1961) or between *+/cr* and *cr/cr* mothers (S ofaer, 1968).

All molar teeth of the normal series were removed from the skulls of four week old mice prepared by papain digestion. The teeth were measured using a projection microscope, a magnified silhouette ($\times 83$) of each tooth being projected onto a graduated screen. The measurements made were the maximum antero-posterior diameters of the molar crowns parallel to the occlusal plane. Corresponding measurements from the right and left sides were pooled.

3. RESULTS

The data are presented as distributions of raw measurements in Fig. 1. There is a clear trend between groups common to measurements for all teeth. Compared with inbred controls, the *+/cr* group is most normal, which is consistent with the recessivity of *cr* for other characters, the *Ta/+* group less so, and the doubly heterozygous group distinctly most abnormal. However, there was an apparently anomalous finding for the lower first molar in that the effect of the mutant genes was to increase rather than reduce the size of this tooth.

Because there is reason to believe that the distributions, at least of first molar measurements, are not strictly homogeneous, statistical procedure was confined to the calculation of means and t values to test the significance of the difference between the mean of the double heterozygotes and that of the *Ta/+* group, the most abnormal single heterozygote group. Since, ideally, the t test requires normality of the distributions being tested, it must be regarded as only a rather gross indicator of significance in the present situation.

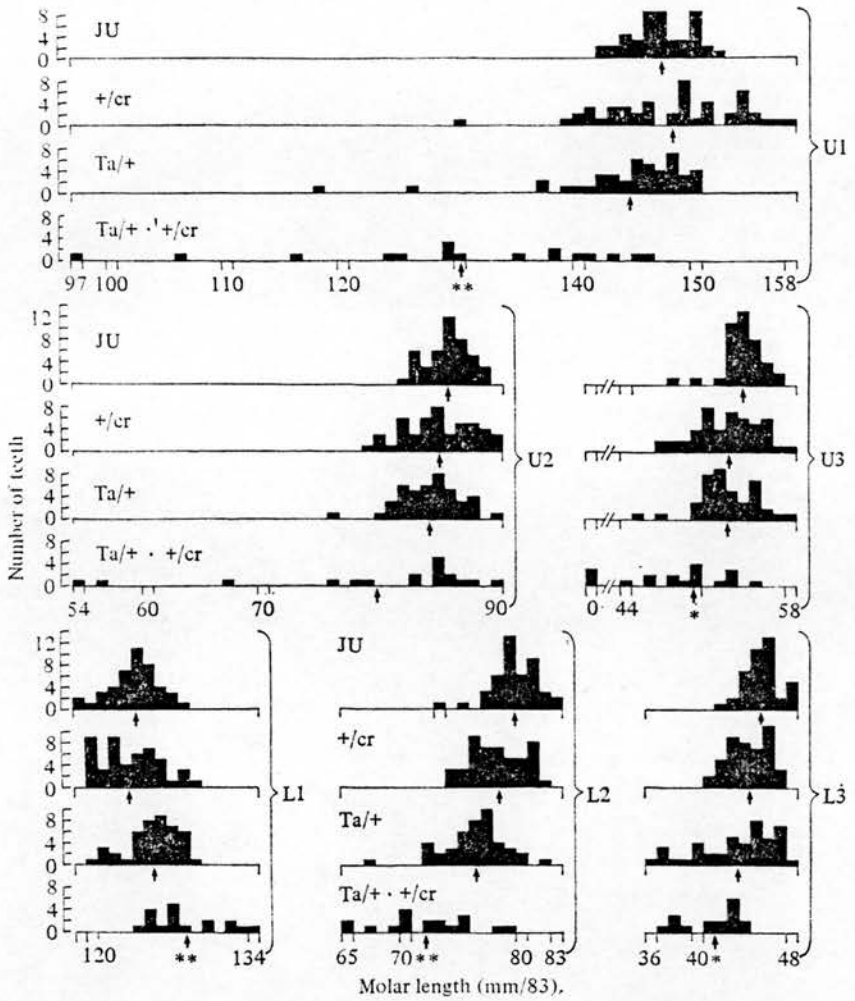


Fig. 1. Distributions of molar length (antero-posterior crown diameter) for the upper 1st, 2nd and 3rd molars (U1, U2, U3) and lower 1st, 2nd and 3rd molars (L1, L2, L3). Arrows show the means of the distributions. A single asterisk indicates a significant difference ($P < 0{\cdot}02$) and a double asterisk a highly significant difference ($P < 0{\cdot}001$) between double heterozygote and *tabby* heterozygote means for each tooth.

The three absent upper third molars in *Ta/+ . +/cr* mice (plotted against zero length) were not included in the calculation of the mean for this group.

4. DISCUSSION

The discovery of mimic genes leads naturally to speculation over the functional relationship between them. Since homozygosity at any one locus alone is sufficient to produce the mutant phenotype, their functions are unlikely to be identical. The simplest explanation is that the wild type allele of each gene is responsible for one of a number of related steps, either in series or in parallel, towards the

formation of an end product or developmental state that is necessary for the production of the normal phenotype. Complete blockage at any point along an isolated pathway is expected to produce the same result. If blockage is incomplete, or if there are cross connexions with other pathways, differences between the genes may be detectable.

Such differences, although not apparent under normal conditions, may be disclosed in different ways: first, by changing genetic background, and second, by various environmental manipulations. Background changes have produced broadly similar modifications of the mutant phenotype for *Ta*, *cr* and *dl*, though certain phenotypic details showed minor differences of response (Sofaer, 1969*b*). A more definite difference between *Ta* and *dl* has been demonstrated with respect to the ability of embryonic tail tissue to produce hair follicles when cultured on the chick chorioallantoic membrane. Downless tails failed to produce follicles, which is in keeping with the adult phenotype of both downless and tabby mice, whereas tabby tails produced follicles at about 40% of the normal control level. Furthermore, dermis–epidermis recombination experiments have demonstrated clearly that the effects of both *dl* and *cr* are restricted to the epidermis, but have not provided similar evidence of a primary epidermal effect for *Ta* (Sofaer, 1974; Mayer, Miller & Green, 1977).

A third way of drawing inferences about the functional relationships between mimic genes is to compare the usual mutant phenotype with that shown by doubly mutant animals. Kindred (1967) investigated the effects on the coat and vibrissae of combinations between *Ta* and *cr*, the genetic backgrounds being those of lines selected for high and low total vibrissa number in tabby mice. Both *Ta* and *cr* normally reduce the number of vibrissae. However, the addition of a crinkled allele increased vibrissa number in both *Ta*/+ mice with the high selection line background and *Ta*/*Y* mice with the low selection line background. In other words, the addition of crinkled in these situations produced a phenotype closer to wild type. By contrast, when *cr* was added to *Ta*/*Y* on the high selection line background, the mutant effect was enhanced. The coat findings were inconclusive. Similar evidence of interaction between *Ta* and *cr* had already been found in other stocks by Fraser, Nay & Kindred (1959) but was then thought to be due to a background effect. These findings are difficult to interpret, though they do suggest the possibility of interaction between the two loci.

The size of molar tooth crowns is a valuable indicator of early developmental conditions since, once the crowns have been formed, they do not alter except as a result of occlusal wear. Such wear does not appreciably affect the dimensions normally studied in younger animals. The present results therefore show that on the *JU*/*Fa* genetic background, at least during the time when the molar crowns develop, the combination of heterozygosity at both the tabby and crinkled loci produces a more extreme mutant phenotype than heterozygosity at either locus alone. For all teeth except the lower first molar the change in phenotype was in the expected direction of reduction in tooth size. The paradoxical increase in size produced by the mutant alleles in the lower first molar was nevertheless consistent

with the findings for the other teeth in that the deviation from wild type was least in the $+/cr$ group, greater for $Ta/+$ and greatest of all for $Ta/+ . +/cr$.

The increase in size of lower first molars may be explained in terms of a difference of developmental timing between upper and lower first molars. The basis of such an explanation is as follows. The mutants appear to have their effect in two main phases characterized by partial suppression of epithelial growth and differentiation. These two phases are separated by a period, from 17 days of gestation to birth, during which there is no suppression. Early growth of the epithelium of the first molar occurs during the first suppression phase, with the majority of its later growth and differentiation occurring when suppression is no longer active. After suppression has been released there appears to be rapid compensatory increase in size, the amount of size increase probably depending on the state of epithelial differentiation. Indeed, in cases where supernumerary teeth develop, it is just this period when they reach a remarkably large size and advanced state of differentiation in a relatively short time (Sofaer, 1969*a*). A slight difference of degree in epithelial differentiation between upper and lower tooth germs at 17 days of gestation could perhaps allow more rapid compensatory increase in size in the lower first molar than the upper, sufficient in some cases actually to result in overcompensation. This phenomenon would not apply to the other molars since the second half of the growth and differentiation of the second molar germ, and the entire development of the third molar germ, occur during the second suppression phase.

The material was collected at the Institute of Animal Genetics, Edinburgh, during 1966/7. The measurements were made recently by Edith Redpath at the Department of Oral Medicine and Oral Pathology.

REFERENCES

BADER, R. S. (1965). A partition of variance in dental traits of the house mouse. *Journal of Mammalogy* **46**, 384–388.

CATTANACH, B. M. (1975). Private communication. *Mouse News Letter* **53**, 29.

CROCKER, A. J. M. (1978). Private communication. *Mouse News Letter* **59**, 20–21.

FALCONER, D. S. (1953). Total sex-linkage in the house mouse. *Zeitschrift für indukt. Abstammungs- und Vererbungslehre* **85**, 210–219.

FALCONER, D. S., FRASER, A. S. & KING, J. W. B. (1951). The genetics and development of 'crinkled', a new mutant in the house mouse. *Journal of Genetics* **50**, 324–344.

FRASER, A. S., NAY, T. & KINDRED, B. M. (1959). Variation of vibrissa number in the mouse. *Australian Journal of Biological Sciences* **12**, 331–339.

GRÜNEBERG, H. (1965). Genes and genotypes affecting the teeth of the mouse. *Journal of Embryology and Experimental Morphology* **14**, 137–159.

GRÜNEBERG, H. (1966*a*). The molars of the *tabby* mouse, and a test of the 'single-active *X*-chromosome' hypothesis. *Journal of Embryology and Experimental Morphology* **15**, 223–244.

GRÜNEBERG, H. (1966*b*). More about the *tabby* mouse and about the Lyon hypothesis. *Journal of Embryology and Experimental Morphology* **16**, 569–590.

GRÜNEBERG, H. (1971). The glandular aspects of the *tabby* syndrome in the mouse. *Journal of Embryology and Experimental Morphology* **25**, 1–19.

KINDRED, B. M. (1961). A maternal effect on vibrissa score due to the *tabby* gene. *Australian Journal of Biological Sciences* **14**, 627–636.

KINDRED, B. M. (1967). The expression of the *tabby* and *crinkled* genes in different genetic backgrounds in the mouse. *Genetics* **55**, 173–178.

MAYER, T. C., MILLER, C. K., & GREEN, M. C. (1977). Site of action of the crinkled (*cr*) locus in the mouse. *Developmental Biology* **55**, 397–401.

ROBERTS, R. C. (1966). Private communication. *Mouse News Letter* **35**, 24.

SOFAER, J. A. (1968). Ph.D. thesis, University of Edinburgh.

SOFAER, J. A. (1969*a*). Aspects of the tabby-crinkled-downless syndrome. I. The development of tabby teeth. *Journal of Embryology and Experimental Morphology* **22**, 181–205.

SOFAER, J. A. (1969*b*). Aspects of the tabby-crinkled-downless syndrome. II. Observations on the reaction to changes of genetic background. *Journal of Embryology and Experimental Morphology* **22**, 207–227.

SOFAER, J. A. (1974). Differences between *tabby* and *downless* mouse epidermis and dermis in culture. *Genetical Research* **23**, 219–225.

SOFAER, J. A. (1975). Interaction between tooth germs and the adjacent dental lamina in the mouse. *Archives of Oral Biology* **20**, 57–61.

SOFAER, J. A. (1977). The teeth of the 'sleek' mouse. *Archives of Oral Biology* **22**, 299–301.

STEVENS, L. C. (1963). Private communication. *Mouse News Letter* **29**, 40.

Journal of Medical Genetics, 1981, **18**, 410–413

Genes for super-intelligence?

JEFFREY A SOFAER* AND ALAN E H EMERY†

*From *the Department of Oral Medicine and Oral Pathology, University of Edinburgh, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR; and †the University Department of Human Genetics, Western General Hospital, Edinburgh EH4 2XU*

SUMMARY The results of a postal questionnaire distributed to British members of Mensa failed to confirm an association of superior intelligence with torsion dystonia, retinoblastoma, or phenylketonuria, but were consistent with real associations between high IQ and infantile autism, gout, and myopia. Further confirmation of these findings in other populations might well indicate that genes producing these disorders have more or less direct effects on cerebral development and function.

It is well known that a number of single genes can influence IQ scores, several inherited malformations and biochemical defects being associated with abnormally low levels of intelligence. Perhaps less well known are recent reports suggesting that among the families of those with certain heritable disorders there is a greater proportion of subjects with unusually high intelligence than could reasonably be expected to occur by chance.[1]

Six disorders for which an association with high IQ has been suggested are recessive torsion dystonia,[2] retinoblastoma,[3 4] phenylketonuria,[5 6] infantile autism,[7] gout,[8] and myopia,[9] though for retinoblastoma[10] and phenylketonuria [11 12] there have been observations inconsistent with the earlier findings. In the present study, an attempt was made to confirm the association between each of the six disorders and unusually high intelligence. This was done not by measuring IQ in families containing affected subjects, as in the past, but by ascertaining the prevalence of the disorders in a sample of highly intelligent subjects and their first degree relatives, and, where possible, by comparing the observed prevalence with published figures for the general population. Because the biochemical basis for some of the disorders is known, and because the biochemistry of all of them may ultimately be understood, such confirmation could help to provide situations in which specific biochemical differences are known to be associated with measurable degrees of enhancement of intellectual ability. This could be a valuable step towards a better understanding of cerebral development and function.

Received for publication 10 December 1980

Subjects and methods

The subjects of the investigation were the approximately 2100 British members of Mensa, the international society for the highly intelligent. Membership of Mensa is only open to people whose IQ score is at least 2 SD above the mean, approximately within the top 2% of the general population. On the Cattell scale (mean 100, SD 24), which is used by Mensa as their criterion for membership, this means that eligibility is restricted to those who score 148 IQ points or more.

Each subject was sent a questionnaire designed to ascertain whether a Mensa member or any first degree relative suffered from any of the six disorders being studied. For torsion dystonia, retinoblastoma, phenylketonuria, and infantile autism a brief description of each disorder was given. Positive responses for these four conditions were noted before the data were analysed and letters sent to the subjects concerned requesting permission to confirm the diagnosis with the hospitals where they or their relatives had been seen. The criterion for myopia was the need to wear glasses for shortsightedness before the age of 10.

Results

Of the 2100 questionnaires distributed, a total of 1355 were returned more or less fully completed, a response rate of 65%. Male respondents totalled 817, females 373, and for the remaining 165 the sex could not be determined because insufficient personal information had been given. The 1355 Mensa members provided information for 5821

first degree relatives: 2647 parents, 1969 sibs, and 1205 children.

There were five positive responses for torsion dystonia, one in a Mensa member and four in relatives. Correspondence revealed that none of these was a case of true torsion dystonia. There were two positive responses for retinoblastoma, both in Mensa members, but for neither was the diagnosis confirmed. The single positive response for phenylketonuria, in the son of a Mensa member, was confirmed by a consultant psychiatrist. The population incidence per 10 000 of recessive torsion dystonia, retinoblastoma, and phenylketonuria can be taken respectively as less than 0·3,[13] 0·3 to 1·0,[14] and 0·6 to 2·0.[15] The results therefore provide no evidence for abnormally high incidence of these disorders among Mensa members or their relatives, although, because of the rarity of the disorders, the sample size may have been inadequate in this respect.

TABLE 1 *No of confirmed cases of infantile autism compared with reported population incidence*[17]

	Autism in sibs and children of respondents		
Population incidence per 10 000	*Observed*	*Per 10 000*	*Relative to general population*
2–4	4/3174	12·6	3·2–6·3

For infantile autism there were ten positive responses, three for Mensa members themselves and seven for their relatives. In only four of these cases, all either sibs or children of respondents, was the diagnosis confirmed by a consultant psychiatrist. Assuming that it is unlikely for a case of infantile autism to become either a parent[16] or a member of Mensa, the incidence of the disorder should be calculated only with respect to sibs and children of respondents. Table 1 shows that this was 3·2 to 6·3 times greater than reported for the general population.

The results for gout are compared with figures for the general population in table 2. The prevalence among male respondents was higher than among brothers of all respondents, and data for both sexes combined show that the prevalence among sibs at mean age 35 was twice that reported for the general population at mean age 44.

The results for myopia are summarised in table 3. Both male and female respondents showed a significantly higher incidence of the disorder than either like-sexed sibs or children of all respondents. The incidence was similar in both sexes and comparable in sibs and children. The lower incidence among parents could perhaps be attributed to less efficient diagnosis of myopia in the parental generation.

TABLE 2 *No of male, female, and all respondents, and like-sexed relatives of all respondents, for whom a positive response for gout was given. Figures for the general population*[18] *are included for comparison*

	Positive responses for gout								
	Males			*Females*			*Both sexes*		
	Mean age	*No*	*%*	*Mean age*	*No*	*%*	*Mean age*	*No*	*%*
Respondents	36	14/817*	1·7	36	5/373†	1·3	36	24/1355‡	1·8
Sibs	34	6/965	0·6	35	5/918	0·5	35	11/1969	0·6
Children	14	0/612	0·0	14	1/533	0·2	14	1/1205	0·1
Parents	64	50/1319	3·8	63	10/1328	0·8	63	60/2647	2·3
General population	—	—	—	—	—	—	44	13/5127	0·3
	58	65/2283	2·8	58	11/2844	0·4	58	76/5127	1·5

*Significantly higher than in brothers of all respondents ($p < 0·05$).
†Not significantly higher than in sisters of all respondents.
‡Not directly comparable with sibs because of unequal sex distribution.

TABLE 3 *No of male, female, and all respondents, and like-sexed relatives of all respondents, who were reported to have required glasses for shortsightedness before the age of 10. Only subjects aged 10 and above are included*

	Required glasses for shortsightedness before age 10					
	Males		*Females*		*Both sexes*	
	No	*%*	*No*	*%*	*No*	*%*
Respondents	149/817*	18·2	85/373*	22·8	270/1355*	19·9
Sibs	101/932	10·8	100/883	11·3	207/1895	10·9
Children	46/400	11·5	41/345	11·9	91/788	11·5
Parents	78/1319	5·9	59/1328	4·4	137/2647	5·2

*Significantly higher than in either like-sexed sibs or children of all respondents ($p < 0·005$).

Unfortunately, it was not possible to compare these incidence figures with figures for the general population because population surveys have generally used different and more technical criteria for defining myopia.

The findings therefore appear to be consistent with real associations between high IQ and infantile autism, gout, and myopia.

Discussion

Two drawbacks of the investigation method must be borne in mind when these results are being interpreted. One concerns selection of the sample, and the other the level of ascertainment in respondents and their relatives.

The sample of respondents was a doubly selected group that consisted of only a very small proportion of all highly intelligent subjects. Firstly, the top 2% of the United Kingdom population exceeds 1 million, so that the entire British membership of Mensa (2100) represents only about 0·2% of those eligible. Secondly, only 65% of the membership responded, and the desire to co-operate may have been biased by whether or not there was something positive to report.The difficulty of sample selection is removed, however, if comparisons are made not between the sample and the general population, but within the sample between respondents and their relatives. This could be done for both gout and myopia, but the possibility of different levels of ascertainment in respondents and their relatives then becomes a potentially confounding factor.

The significantly greater prevalence of gout in male respondents than in brothers of all respondents could have been at least partly the result of a lower level of ascertainment for relatives, but since the prevalence among sibs of both sexes was rather high compared with the general population, under-reporting for relatives would imply an even higher prevalence among brothers. As far as myopia is concerned, Mensa members can be expected to have responded accurately on their own children's need to wear glasses before the age of 10. The fact that the observed incidence was similar in both sibs and children therefore suggests that under-reporting made little or no contribution to the difference between respondents and sibs for this disorder. An ascertainment effect is therefore unlikely to explain the association between high IQ and either gout or myopia in the present sample.

There are several ways in which superior intelligence and disease may be associated. An association would occur if a disorder were more readily diagnosed in brighter families. There may be a cause and effect relationship, as has been postulated for myopia, shortsightedness possibly developing as a result of high IQ through reading to excess. Fortuitous associations could result from the chance occurrence of an unusually high incidence of a disorder in brighter families. However, because a substantial genetic component appears to be involved in the aetiology of infantile autism, gout, and myopia,[19–21] and because IQ is also to some extent inherited,[22] there may be purely genetic explanations for the observed associations. The most attractive of these is pleiotropy, namely that the genes producing the disorders have more or less direct effects on cerebral development and function. Associations for other genetic reasons such as linkage, or through chance, are unlikely to be maintained when samples from several different sources are studied. Therefore, assuming that appropriate allowance can be made for non-genetic effects, the greater the variety of samples in which the same association is observed, the greater the likelihood of pleiotropy.

Independent evidence for pleiotropy may be forthcoming if the biochemistry of each disorder is at least partly understood. At present this applies only to gout, in connection with which some interesting observations and speculations have been made. Among mammals, significant levels of serum uric acid are found only in higher apes and man, other mammals possessing the enzyme uricase which oxidises uric acid to allantoin. It has been suggested that uric acid, like other purines, can stimulate the cerebral cortex, and that the superior intellectual powers of the higher primates may be to some extent a consequence of high uric acid levels through mutations causing loss of uricase activity.[23] Consistent with this idea is the finding that glutamic acid, which is involved in the production of endogenous uric acid,[24] appears to improve cognitive function when given therapeutically in cases of mental retardation.[25]

It is possible that future investigations will further confirm the association between various inherited disorders and superior intelligence, and that, as the biochemistry of the disorders becomes better understood, there may be direct demonstrations of intellectual enhancement by the products of the genes involved.

The authors are grateful to the members and administration of Mensa for their co-operation; to Mr W Lutz, Director of Edinburgh University's Medical Computing and Statistics Unit, for advice on questionnaire design; and to the International Brain Research Organisation, Mason Medical Research Foundation, and W R Henderson's Trust for financial support.

References

1 Anonymous. Genes for superior intelligence. *Br Med J* 1976;i:416–7.

2 Eldridge R, Harlan A, Cooper IS, Riklan M. Superior intelligence in recessively inherited torsion dystonia. *Lancet* 1970;i:65–7.

3 Thurrell RJ, Josephson TS. Retinoblastoma and intelligence. *Psychosomatics* 1966;**7**:368–70.

4 Williams M. Superior intelligence of children blinded from retinoblastoma. *Arch Dis Child* 1968;**43**:204–10.

5 Munro TA. Phenylketonuria: data on forty-seven British families. *Ann Eugen* 1947;**14**:60–88.

6 Fuller R. Phenylketonuria: psychological and developmental evaluation. In: Anderson JA, Swaimson KF, eds. *Phenylketonuria and allied metabolic diseases*. Washington: US Government Printing Office, 1967:133–51.

7 Rimland B. *Infantile autism*. London: Methuen, 1965.

8 Anonymous. Uric acid and the psyche. *JAMA* 1969;**208**: 1180.

9 Karlsson JL. Influence of the myopia gene on brain development. *Clin Genet* 1975;**8**:314–8.

10 Eldridge R, O'Meara K, Kitchin D. Superior intelligence in sighted retinoblastoma patients and their families. *J Med Genet* 1972;**9**:331–5.

11 Thalhammer O, Havelec L, Knoll E, Wehle E. Intellectual level (IQ) in heterozygotes for phenylketonuria (PKU). *Hum Genet* 1977;**38**:285–8.

12 Ford RC, Berman JL. Phenylalanine metabolism and intellectual functioning among carriers of phenylketonuria and hyperphenylalaninaemia. *Lancet* 1977;i:767–71.

13 Eldridge R, Edgar A, Cooper IS. Genetics, geography and intelligence in the torsion dystonias. *Birth Defects* 1971; VII:167–77.

14 Vogel F. Genetics of retinoblastoma. *Hum Genet* 1979; **52**:1–54.

15 Tourain AY, Sidbury JB. Phenylketonuria. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. *The metabolic basis of inherited disease*. 4th ed. New York: McGraw-Hill, 1978:240–55.

16 Rutter M. Autistic children: infancy to adulthood. *Semin Psychiatry* 1970;**2**:435–50.

17 Wing L, Yeates SR, Brierly LM, Gould J. The prevalence of early childhood autism: comparison of administrative and epidemiological studies. *Psychol Med* 1976;**6**:89–100.

18 Hall AP, Barry PE, Dawber TR, McNamara M. Epidemiology of gout and hyperuricemia: a long term population study. *Am J Med* 1967;**42**:27–37.

19 Folstein S, Rutter M. Genetic influences and infantile autism. *Nature* 1977;**265**:726–8.

20 Morton NE. Genetics of hyperuricemia in families with gout. *Am J Med Genet* 1979;**4**:103–6.

21 Karlsson JL. Evidence for recessive inheritance in myopia. *Clin Genet* 1975;**7**:197–202.

22 Block N, Dworkin G. *The IQ controversy*. London: Quartet Books, 1977.

23 Orowan E. The origin of man. *Nature* 1955;**175**:683–4.

24 Gutman AB, Yu TF. Uric acid metabolism in normal man and in primary gout. *N Engl J Med* 1965;**273**, 252–60.

25 Vogel W, Brovermen DM, Draguns JG, Klaiber EL. The role of glutamic acid in cognitive behaviours. *Psychol Bull* 1966;**65**:367–82.

Requests for reprints to Dr J A Sofaer, Department of Oral Medicine and Oral Pathology, University of Edinburgh, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR.

DENTAL EXTRACTIONS IN PAGET'S DISEASE OF BONE

Running title: EXTRACTIONS IN PAGET'S DISEASE

J.A. Sofaer

University of Edinburgh,

Department of Oral Medicine and Oral Pathology

and

Department of Human Genetics

Abstract

The results of a postal questionnaire completed by 360 patients with Paget's disease of bone, on behalf of themselves and their unaffected spouses, suggest that dental practitioners have some awareness of the potential problems associated with extractions for patients with Paget's disease, but that nevertheless patients still suffer from greater difficulty at extraction and more post-extraction complications than normal.

Introduction

Paget's disease of bone is a remarkably common condition in later life, characterised by rapid remodelling and the development of structurally abnormal bone. It can be the cause of pain, fracture, deformity and rarely malignancy. Radiological surveys have detected bony changes consistent with the disease in 2.3 - 8.3 per cent of the British population at age 55 years and above (1), with prevalence rates for native Australians (4) and for both blacks and whites in the United States (5) falling around the lower end of this range. Symptoms are relatively uncommon, but because of its high prevalence the disease still makes a considerable contribution to morbidity among the elderly. The aetiology of Paget's disease is unknown, but evidence is accumulating to suggest that it may be caused by a slow virus infection (7, 14).

The jaws are thought to be involved only infrequently in Paget's disease. Dental radiographs of 138 patients have revealed evidence of the disease in the maxilla of 20 patients and in the mandible of 3 patients (12). A more recent survey has shown that of 1225 patients with Paget's disease as an incidental finding on routine radiology, 27 per cent showed skull involvement; but of these, radiographic changes could be found in the maxilla of only 2 patients and in the mandible of only 1 patient (6).

It is generally accepted that dental extractions for patients with Paget's disease of the jaws are likely to be more difficult and followed by more complications than normal. In the early stages of the disease the bone is unusually vascular, with the risk of excessive post-extraction haemorrhage, while later there is progressive sclerosis predisposing to healing complications and infection. Furthermore, there may be hypercementosis and/or ankylosis of the roots (8).

Difficulties and complications associated with extractions for Paget's disease patients have been mentioned in a number of previous reports (2,3,9,10). However, conclusions have generally been based on small selected samples or single case reports. By contrast, the present study makes use of a large sample of Paget's disease patients to assess both the dental profession's awareness of potential problems associated with extraction and the frequency with which extraction difficulties and complications arise irrespective of the accepted clinical or radiographic signs of jaw involvement.

Subjects and methods

As part of a large family study of Paget's disease, 520 known cases of the disease, almost all of whom were ascertained through the British National Association for the Relief of Paget's Disease, were sent a postal questionnaire requesting dental extraction history information about themselves and their living spouses. Of the 520 questionnaires distributed 360 were returned completed, a response rate of 69 per cent.

Respondents were first asked to confirm whether or not they or their spouses suffered from Paget's disease. For each Paget's disease patient and for each spouse, each of the seven questions listed in Table 1 was then asked four times:

first, for upper extractions carried out at age 40 years or younger;
second, for lower extractions - - - - " - - - -
third, for upper extractions carried out at age 41 years or older;
fourth, for lower extractions - - - - " - - - -

Answers to questions 2 to 4 should reflect the dental profession's attitude towards extraction for Paget's disease patients, and answers to questions 5 to 7 should provide some indication of the overall frequency of difficulties and complications. With reference to question 7, respondents were also asked to indicate the nature of any complication that occurred.

Numbers of positive and negative responses were tabulated, and the percentages of positive responses were calculated. Comparisons were then made by sex, by age at extraction, by jaw, and by presence versus absence of Paget's disease, using 2 x 2 chi-square tests with the numbers of individuals involved in each positive and negative category.

Results

All respondents confirmed that they suffered from Paget's disease, and all spouses were unaffected. The numbers of Paget's disease patients and normal spouses, together with their mean ages at the time of completing the questionnaire, are given in Table 2.

Tables 3 and 4 summarise responses to question 1. Table 3 shows that for both Paget's patients and normal spouses, a higher proportion of individuals reported extractions at or before age 40 than at or after age 41. Also, a significantly higher proportion of Paget's patients than of normal spouses reported extractions, both overall, and for each sex alone at or before age 40. Table 4 shows that among Paget's patients, for extractions carried out at or before age 40, upper extractions were significantly more common than lower extractions

for females and for both sexes combined. This difference between jaws was not found for normal spouses. Upper extractions were also significantly more common among Paget's patients than among normal spouses, again both for females and for both sexes combined.

Figure 1 summarises comparisons by sex, by age at extraction and by presence versus absence of Paget's disease for questions 2 to 7. Comparisons by jaw are not included because none achieved statistical significance. Responses to question 5 (difficulty at extraction) have been subdivided into 5a (all extractions) and 5b (only those for which local anaesthesia was given). Of the comparisons by sex (Fig. 1A) only one achieved statistical significance, a higher proportion of female as opposed to male Paget's patients having had a general anaesthetic (question 4). Of the comparisons by age at extraction (Fig. 1B) only two showed significant differences, both among Paget's patients. Extractions at or after age 41 were more often preceded by an X-ray examination than extractions at or before age 40 (question 3); and extractions carried out in the younger age range showed a higher frequency of heavy or prolonged bleeding than those in the older age range, the frequency at or after age 41 in Paget's patients being comparable to that reported by normal spouses for extractions in either age range (question 6). The frequency of positive responses among Paget's patients relative to normal spouses (Fig. 1C) showed the same pattern among females and males with the exception of questions 3 and 4. Paget's patients reported significantly more frequent X-ray examination prior to extraction (males, question 3), general anaesthesia (females, question 4), difficulty at extraction (males, questions 5a and 5b) and post-extraction complications (both

sexes, question 7). The majority of the complications described were consistent with 'dry socket'.

Discussion

There are a number of difficulties involved in a survey of this type, particularly with regard to the accuracy of the information given, and to whether respondents are representative of Paget's disease patients in general. The diagnostic criteria for Paget's disease in the sample cannot be defined, but because the majority of those who have radiological signs of the disease are symptomless, it can be assumed that respondents, all of whom knew they had the disease, occupied the more severe end of the Paget's disease spectrum. Similarly, some of the presumed normal spouses, if examined radiologically, might have shown early signs of the disease. However, these difficulties are minimised if comparisons are made within the sample only, that is between respondents and their living spouses.

A greater proportion of Paget's disease patients than normal spouses reported that they had undergone dental extraction, particularly at age 40 or younger (Table 3). This is perhaps attributable to loosening of the teeth resulting from changes in the supporting bone, as well as to occasional root resorption (2,11). The greater frequency of upper as opposed to lower extractions in

Paget's patients (Table 4) would then be consistent with the more frequent involvement of the maxilla (2,12,13).

Questions 2 to 4 showed some evidence of greater frequency of positive responses for Paget's patients compared with normal spouses (Fig. 1C), suggesting that the dental profession tends to anticipate some difficulty with extractions for patients with Paget's disease. A higher frequency of general anaesthesia is assumed to be due to relative reluctance to use local anaesthetics containing vasoconstrictors in the presence of sclerotic bone, to allow the healing socket a better chance of remaining infection free. For questions 5 to 7, respondents showed consistently higher frequencies of positive responses compared with normal spouses, although not all comparisions were significant (Fig. 1C), suggesting that Paget's disease patients in general are indeed more likely than normal to suffer from difficulty at extraction and from post-extraction complications. The greater frequency of heavy or prolonged bleeding following extractions in the younger compared with the older age range for Paget's patients (Fig. 1B) is consistent with initial vascularity and progressive sclerosis of the supporting bone.

The results are in keeping with the known skeletal distribution and progress of Paget's disease. They also suggest that dental practitioners have some awareness of the potential problems associated with extractions for patients with Paget's disease, but that nevertheless patients still tend to suffer from greater difficulty at extraction and more post-extraction complications than normal. Perhaps this is inevitable, but on the other hand it may be that there is some room for improvement in the management of dental extractions in Paget's disease. Against the background of

rarity of obvious jaw involvement in unselected samples, the results suggest that even Paget's disease patients without the accepted clinical or radiographic evidence of jaw disease may be unusually prone to extraction problems. However, proof of this last point would require more detailed investigation.

Acknowledgements

This survey was carried out as part of a large family study supported by a grant from the British National Association for the Relief of Paget's Disease to Professor A.E.H. Emery. The author is grateful to Professor J.C. Southam for advice on planning the questionnaire and to Mrs Loraine Williamson for clerical assistance.

Table 1. The seven questions relating to extraction history and the two categories into which responses were divided for analysis.

Question	Responses	
	Negative	Positive
1. Have you had any teeth extracted?	No	Yes
2. Where were the teeth extracted?	Own GDP only	Hospital/Clinic/ GDP
3. Were any X-rays taken before the teeth were extracted?	No	Yes
4. What type of anaesthetic was given?	Local only	General/Local
5. Would you say that the extractions were surprisingly easy, normal or unusually difficult?	Easy/Normal	Difficult
6. Following the extractions would you say that bleeding was less than expected, normal or unusually heavy or prolonged?	Less than expected/ Normal	Heavy or prolonged
7. Would you say that healing of the tooth sockets was uneventful or complicated by problems?	Uneventful	Complicated

Table 2. Number and mean age (in years) of Paget's disease patients and their normal spouses.

	Females		Males		Both sexes	
	Number	Mean age	Number	Mean age	Number	Mean age
Paget's patients	225	72	135	71	360	72
Normal spouses	113	67	135	73	248	70

Table 3. Per cent Paget's disease patients and normal spouses who reported extractions from either jaw at or before age 40 years ($\leq$ 40), at or after age 41 years ($\geq$ 41) and at any age (*p $\leq$ 0.05, **p $\leq$ 0.01).

	Females			Males			Both sexes
	$\leq$ 40	$\geq$ 41	p	$\leq$ 40	$\geq$ 41	p	(Any age)
Paget's patients	91	73	**	88	74	**	99
Normal spouses	79	66	*	77	65	-	95
p	**	-		*	-		**

Table 4. Per cent Paget's disease patients and normal spouses who reported upper and lower extractions at or before age 40 years (*$p \leq 0.05$, **$p \leq 0.01$).

	Females			Males			Both sexes		
	Upper	Lower	p	Upper	Lower	p	Upper	Lower	p
Paget's patients	87	78	*	83	79	-	85	78	*
Normal spouses	71	73	-	74	70	-	73	71	-
p	**	-		-	-		**	-	

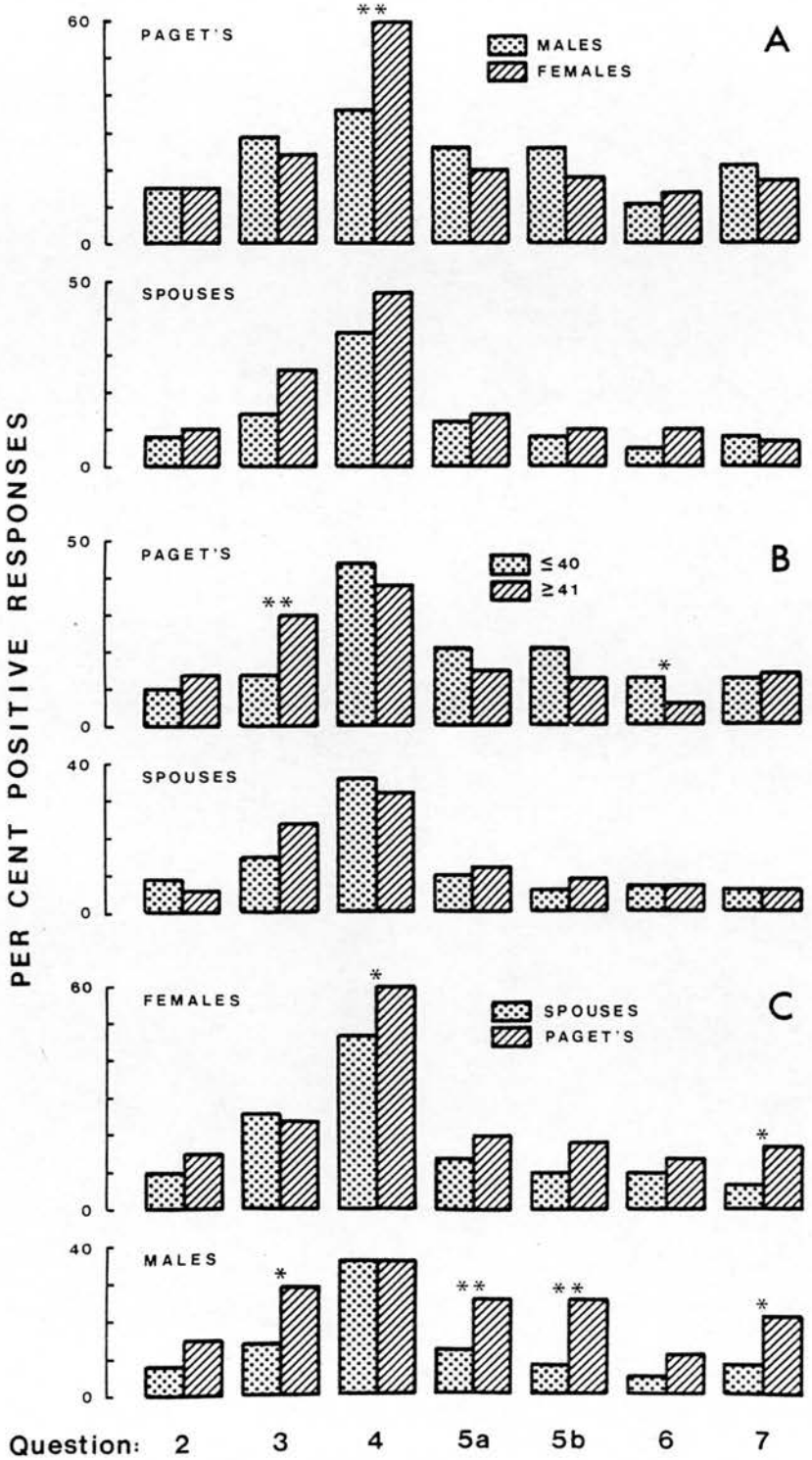


Figure 1. Per cent positive responses: A, by sex (extractions at any age from either jaw); B, by age at extraction (extractions for both sexes from either jaw); C, Paget's disease versus normal spouses (extractions at any age from either jaw).

*$p \leq 0.05$ **$p \leq 0.01$

References

1. BARKER, D.J.P., CHAMBERLAIN, A.T., GUYER, P.B. & GARDNER, M.J.: Paget's disease of bone: the Lancashire focus. BMJ, 1980: 1: 1105-1107.

2. COOKE, B.E.D.: Paget's disease of the jaws: fifteen cases. Ann R Coll Surg Engl. 1956: 19: 223-240.

3. FEIG, H.I., EDUMUNDS, W.R., BEAUBIEN, R. & FINKELMAN, A.A.: Chronic osteomyelitis secondary to Paget's disease: a complication following dental extraction. Oral Surg. 1969: 28: 320-325.

4. GARDNER, M.J., GUYER, P.B. & BARKER, D.J.P.: Radiological prevalence of Paget's disease of bone in British migrants to Australia. BMJ, 1978: 1: 1655-1657.

5. GUYER, P.B. & CHAMBERLAIN, A.T.: Paget's disease of bone in two American cities. BMJ, 1980: 1: 985.

6. GUYER, P.B. & CLOUGH, P.W.L.: Paget's disease of bone: some observations on the relation of the skeletal distribution to pathogenesis. Clin Radiol. 1978: 29: 421-426.

7. HOSKING, D.J.: Paget's disease of bone. BMJ, 1981: 2: 686-688.

8. MACDONALD, D.G. & LINDSAY, R.: Skeletal disease. In: JONES, J.H. & MASON, D.K. (eds.): Oral manifestations of systemic disease. W.B. Saunders, London 1980.

9. McGOWAN, D.A.: Clinical problems in Paget's disease affecting the jaws. Br J Oral Surg. 1974: 11: 230-235.

10. RIPP, G.A.: A complication after extraction in a patient with advanced Paget's disease. Oral Surg. 1972: 33: 35-40.

11. SMITH, N.H.H.: Monostotic Paget's disease of the mandible presenting with progressive resorption of the teeth. Oral Surg. 1978: 46: 246-253.

12. STAFNE, E.C. & AUSTIN, L.T.: A study of dental roentgenograms in cases of Paget's disease (osteitis deformans), osteitis fibrosa cystica and osteoma. J. Am. Dent. Ass. 1938: 25: 1202-1214.

13. TILLMAN, H.H.: Paget's disease of bone. Oral Surg. 1962: 15: 1225-1234.

14. WILLIAMS, N.J. (ed.): Diphosphonates and Paget's disease. Br J Clin Pract. 1981: Symposium Supplement 13.

CRITIQUES

The first of the two critiques in this section comments on the application of the Hardy-Weinberg law to population data in an attempt to demonstrate single gene control of various human dental morphological characteristics. This approach is shown to be invalid by a consideration of the principles involved and by the use of an obviously fallacious example. The second critique discusses Jackson's complex theory of caries aetiology, which has been used to account for patterns of site attack by dental caries. It is pointed out that the theory is based on several improbable assumptions for which no independent evidence has been forthcoming. An alternative hypothesis involving a simple quantitative model in keeping with the current majority view of caries aetiology is proposed.

Reprinted from JOURNAL OF DENTAL RESEARCH
Vol. 49, No. 6, November-December 1970

Dental Morphologic Variation and the Hardy-Weinberg Law

J. A. SOFAER

Human Genetics Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014, USA

Recently, the Hardy-Weinberg Law has been applied to population data to support the contention that various human dental morphologic characteristics are controlled by single autosomal genes. By a discussion of the principles involved and by the use of an example, this approach is shown to be invalid.

Recently, attempts have been made to support the contention that various human dental morphologic characteristics are controlled by single autosomal genes, merely by an examination of the frequencies in populations of different rather arbitrarily defined manifestations of each characteristic.[1–3] No reference to individual relationships within the populations were made, and yet conclusions were drawn about the way in which the characteristics are transmitted from one generation to the next. Such conclusions can only be valid if they are drawn from family data, because the study of inheritance clearly demands the use of related individuals. Furthermore, because different modes of inheritance may appear similar, and because nongenetic causes may result in observations that are compatible with some form of genetic control, large samples composed of several different sorts of relative are required.[4,5] In this paper the fallacy of the population approach is discussed in the hope that future investigations will be based on firmer theoretical foundations.

Dental Morphologic Variation

The following characteristics have been considered in the population approach: the cusp of Carabelli (on the lingual surface of the mesiolingual cusp of the maxillary molars), the hypocone (or distolingual cusp) of the maxillary second molar, the protostylid cusp (on the buccal surface of the mesiobuccal cusp of the mandibular molars), and shovel-shaped incisors. The level of manifestation of each characteristic is variable, and the incidence of each may differ from one population to another.[6–14] All are quasi-continuous variables; ie, they are either present or absent, but when present they vary continuously from the lowest level of expression to the highest.

The accepted model of quasi-continuous variation is based on the assumption that there is an underlying scale of continuous variation of some attribute (a combination of all the genetic and environmental factors involved) that is immediately related to the development of the character. The character is absent in individuals below the threshold level and present in those above it. The more the level on the underlying scale exceeds the threshold, the more intense the expression of the character is likely to be. A quasi-continuous character can therefore be regarded as a continuous variable whose expression has a "visible" and a "nonvisible" range.[15,16]

Although dental morphologic characters behave as quasi-continuous variables, there is a difficulty involved in analysis that does not apply to a truly metric quasi-continuous character, eg, third molar size in mice.[15–19] In this situation, any size of tooth can be recorded; the only limitation is the accuracy of the instrument and method used. Precise measurement of a morphologic characteristic is not a simple matter, even though it may be obvious that the degree of its expression varies continuously. It has been necessary, therefore, to use limited and subjective classifications, such as "moderate" and "pronounced" expression, which impose restrictions on the amount of information that can be derived, even from an appropriately designed animal experiment.[20] Fur-

Received for publication December 8, 1969.

thermore, the subjectivity renders data collected by different individuals of "dubious comparative value."[21]

In explanations of the genetic control of human dental morphologic variation in terms of single autosomal genes, the expression of each character has been divided into three classes: nonaffected, minimally to moderately affected, and moderately to maximally affected. This procedure is valid from the phenotypic point of view; but since all three classes appear to represent ranges on the same continuous scale there is, at present, no reason to suspect any qualitative biologic difference between them. However, the assumption has been made in these explanations that the three classes of expression correspond to three distinct genotypes produced by the segregation of two alleles (eg, A_1 and A_2) at a single autosomal locus. All nonaffected individuals have been presumed homozygous for one allele (eg, A_1A_1); minimally to moderately affected individuals have been presumed heterozygous (A_1A_2); and moderately to maximally affected individuals have been presumed homozygous for the other allele (A_2A_2). The agreement of the frequencies of these three hypothetical genotypes in a population with the expectation derived from the application of a fundamental theorem of population genetics, the Hardy-Weinberg Law, has then been taken as evidence of single gene control.[1–3]

Principles of the Hardy-Weinberg Law

Before proceeding further it is important to consider the principles embodied in the Hardy-Weinberg Law. If it is assumed that in a given population p and q are the frequencies of alleles A_1 and A_2 at a single autosomal locus, and that $p + q = 1$, then, if the population is infinitely large, if mating within it is at random, and with certain other reservations, all of which constitute "ideal" conditions, the three genotypes A_1A_1, A_1A_2, and A_2A_2 will appear in the proportions p^2, $2pq$, and q^2, respectively.[22] When the ideal conditions do not apply the genotypic proportions will approximate the expectation.

If a, b, and c are the numbers of individuals of the three genotypes A_1A_1, A_1A_2, and A_2A_2 respectively, and $a + b + c = N$, then, because N individuals possess $2N$ alleles at an autosomal locus, the frequencies of the alleles are $p = (2a + b)/2N$ and $q = (2c + b)/2N$.

Thus, it is a simple matter to estimate p and q, and therefore the Hardy-Weinberg expectation of p^2, $2pq$, and q^2 for the three genotype frequencies from the observed numbers in each category. The chi-square test can then be used to compare the observed numbers of each genotype with those predicted by the Hardy-Weinberg Law.

Criticisms of the Use of the Hardy-Weinberg Law

The fallacy of the approach under consideration lies in the misconception that the appearance in a population of three arbitrarily defined classes in the proportions p^2, $2pq$, and q^2 constitutes evidence of single gene control. As already pointed out, there is no good reason to suppose that

TABLE 1
WAYS OF BREAKING DOWN SAMPLE INTO THREE PARTS AND PERCENTAGE THAT FIT THE HARDY-WEINBERG EXPECTATION AT 5% LEVEL

Sample Size	No. of Ways of Breaking Down into Three Parts	Percentage Fitting Hardy-Weinberg Expectation (to nearest whole no.) at the 5% Level of Significance
25	325	50
50	1,275	36
75	2,850	30
100	5,050	26
200	20,100	18
500	125,250	12

TABLE 2
PERCENTAGE DISTRIBUTION OF ADULT MALE DENTAL PATIENTS IN TWO INCOME GROUPS ACCORDING TO DENTAL VISIT DATA[24]

Annual Income	No. of Patients	Length of Time Since Last Visit to Dentist		
		1 Year and Under	2-9 Years	10 Years and Over or Never
< $1,000	597	45.5	46.4	8.1
$3,000 and over	533	72.6	24.3	3.1

these classes represent genotypes, and if there were, this test would certainly add nothing further. The chi-square test for goodness of fit with the Hardy-Weinberg expectation is concerned only with whether the numbers of each of the three types of individual are distributed according to the binomial expansion. It is clearly incapable of providing any information about the nature of each of these three types of individuals. The test is only meaningful if it is applied to a known locus, identified through a study of related individuals, when its purpose is to see how closely a population resembles the ideal. Even then, the conclusions that can be drawn are limited.[23]

In fact, distribution according to the binomial expansion can occur surprisingly frequently purely by chance. The figures* presented in Table 1 show that there are 325 possible ways of breaking down a sample of 25 into three parts, and about half of these 325 ways fit the Hardy-Weinberg expectation at the 5% level. In a sample of 19 individuals, which has been used in one instance to provide evidence for single gene control of the cusp of Carabelli,[2,3] the probability of fitting the Hardy-Weinberg expectation at the 5% level is therefore greater than 0.5, no matter what the basis is for the distribution of the different levels of expression.

To illustrate that it is not meaningful to invoke a single gene to explain the fit of population frequencies with the Hardy-Weinberg expectation, the method will be applied to data on visiting the dentist, a characteristic that is obviously greatly, if not completely, dependent on social and cultural background.

Table 2 shows the percentage distribution of adult male dental patients for two income groups in three categories according to the time elapsing since their last visit to a dentist.[24] If the character "dental visiting" is controlled by the segregation of two alleles D_v (dental visitor) and D_n (dental nonvisitor) at a single autosomal locus, then individuals for whom the time elapsing since their last dental visit was one year and under could be presumed D_vD_v homozygotes; those for whom the time elapsing

TABLE 3

COMPARISON OF OBSERVED AND EXPECTED PRESUMED GENOTYPE FREQUENCIES

Income Group	Observed No. of Presumed Genotypes			Presumed Allele Frequencies		Expected No. of Presumed Genotypes			χ^2_1*	P
	D_vD_v	D_vD_n	D_nD_n	D_v	D_n	D_vD_v	D_vD_n	D_nD_n		
<$1,000	272	277	48	0.687	0.313	282	257	58	3.64	>0.05
$3,000 and over	387	130	16	0.848	0.152	383	138	12	1.84	>0.10

Individuals for whom the time elapsing since the last dental visit was one year or less are presumed homozygous dental visitors (D_vD_v), those for whom the time elapsing was two to nine years are presumed heterozygous (D_vD_n), and those for whom the time elapsing was ten years and over or who had never visited the dentist are presumed homozygous dental nonvisitors (D_nD_n).

* Chi-squared has one degree of freedom because the expectation is derived from the observations through the computed allele frequencies and the Hardy-Weinberg assumptions.

* P. L. Workman, Division of Medical Genetics, Mt. Sinai School of Medicine, New York, NY 10029, USA.

was two to nine years could be presumed D_vD_n heterozygotes; and those for whom the time elapsing was ten years and over, or who had never visited a dentist, could be presumed D_nD_n homozygotes. Table 3 shows the observed numbers of each presumed genotype, the presumed allele frequencies, and the numbers predicted by an application of the Hardy-Weinberg Law. For both income groups the observations are not significantly different from the expectation at the 5% level. Also, there is a highly significant difference between the two income groups. It would surely be ridiculous to conclude that visiting the dentist is controlled by two alleles at a single autosomal locus, and that the frequency of the dental visitor allele is related to economic status.

Conclusions

It should be reemphasized that the nature of the genetic control of any characteristic can only be derived from a study of related individuals. In a population where relationships are not known or not taken into account, the Hardy-Weinberg Law can only be applied to known loci. The distribution of characters with already established modes of inheritance can then be used to provide information on the genetic structure of the population. It is hoped that the present discussion and fallacious example will serve to indicate that the approach under consideration is not a valid one, and that considerable caution should be exercised in the interpretation of dental variation.

References

1. DEVOTO, F.C.H.; ARIAS, N.H.; RINGUELET, S.; and PALMA, N.H.: Shovel-Shaped Incisors in a Northwestern Argentine Population, *J Dent Res* **47**:820-823, 1968.
2. TURNER, C.G.: Dental Genetics and Microevolution in Prehistoric and Living Koniag Eskimo, *J Dent Res* **46**(suppl): 911-917, 1967.
3. TURNER, C.G.: Microevolutionary Interpretations from the Dentition, *Amer J Phys Anthrop* **30**:421-426, 1969.
4. DAHLBERG, G.: Genetic Investigations in Different Populations, *Acta Genet (Basel)* **3**:117-142, 1952.
5. LILIENFELD, A.M.: A Methodological Problem in Testing a Recessive Genetic Hypothesis in Human Disease, *Amer J Pub Health* **49**:199-204, 1959.
6. CARBONELL, V.M.: Variations in the Frequency of Shovel-Shaped Incisors in Different Populations, in BROTHWELL, D.R. (ed): *Dental Anthropology,* New York: Pergamon Press, 1963.
7. DAHLBERG, A.A.: The Evolutionary Significance of the Protostylid, *Amer J Phys Anthrop* **8**:15-25, 1950.
8. DAHLBERG, A.A.: The Dentition of the American Indian, in LAUGHLIN, W.S. (ed): *Papers on the Physical Anthropology of the American Indian,* New York: Viking Fund, 1951.
9. DAHLBERG, A.A.: Analysis of the American Indian Dentition, in BROTHWELL, D.R. (ed): *Dental Anthropology,* New York: Pergamon Press, 1963.
10. DIETZ, V.: A Common Dental Morphotropic Factor, the Carabelli Cusp, *JADA* **31**:784-789, 1944.
11. HRDLIČKA, A.: Shovel-Shaped Teeth, *Amer J Phys Anthrop* **3**:429-465, 1920.
12. KRAUS, B.S.: Carabelli's Anomaly of the Maxillary Molar Teeth, *Amer J Hum Genet* **3**:348-355, 1951.
13. KRAUS, B.S.: Occurrence of the Carabelli Trait in Southwest Ethnic Groups, *Amer J Phys Anthrop* **17**:117-123, 1959.
14. MOORREES, C.F.A.: *The Aleut Dentition,* Cambridge, Mass: Harvard University Press, 1957.
15. GRÜNEBERG, H.: Genetical Studies on the Skeleton of the Mouse. IV. Quasi-Continuous Variations, *J Genet* **51**:95-114, 1952.
16. GRÜNEBERG, H.: *The Pathology of Development,* Oxford: Blackwell, 1963.
17. GRÜNEBERG, H.: The Genetics of a Tooth Defect in the Mouse, *Proc Roy Soc B* **138**: 437-451, 1951.
18. DEOL, M.S., and TRUSLOVE, G.M.: Genetical Studies on the Skeleton of the Mouse. XX. Maternal Physiology and Variation in the Skeleton of C57BL, Mice *J Genet* **55**:288-312, 1958.
19. SEARLE, A.G.: Genetical Studies on the Skeleton of the Mouse. XI. The Influence of Diet on Variation Within Pure Lines, *J Genet* **52**:413-424, 1954.
20. SOFAER, J.A.: The Genetics and Expression of a Dental Morphological Variant in the Mouse, *Arch Oral Biol* **14**:1213-1225, 1969.
21. BROTHWELL, D.R.: Some Problems and Objectives Related to the Study of Dental Variation in Human Populations, *J Dent Res* **46**(suppl):938-941, 1967.
22. FALCONER, D.S.: *Introduction to Quantitative Genetics,* London: Oliver and Boyd, 1964.
23. WORKMAN, P.L.: The Analysis of Simple Genetic Polymorphisms, *Hum Biol* **41**:97-114, 1969.
24. Economics Committee of the American Dental Association: *A Study of the Dental Needs of Adults in the United States,* Chicago: American Dental Association, 1940.

APRIL 20 1982

Genetics and Site Attack in Dental Caries

Comments on Jackson's Theory

Br Dent J 1982; 152: 267

J. A. SOFAER*, PhD, BDS

Jackson's complex theory of caries aetiology has been used in a number of publications over recent years to account for patterns of site attack by dental caries. However, the theory is based on several improbable assumptions for which no independent evidence has been forthcoming. An alternative hypothesis, involving a simple quantitative model in keeping with the current majority view of caries aetiology, can be used to explain why Jackson's single/double, unilateral/bilateral and asymmetrical/symmetrical attack ratios stabilise with age at levels above zero.

OVER the past few years there have been several reports concerned with the pattern of carious attack on the mesial, distal and lingual pit sites of permanent maxillary incisors[1-7], on the cervical sites of permanent maxillary incisors[4], on the mesial and distal surfaces of permanent mandibular incisors[9-12], on permanent molars[13] and in the permanent dentition as a whole[14]. Major emphasis has been given to the numbers of individuals in whom either one or both members of a pair of maxillary incisor sites have been decayed or filled, the majority of pairs being of 3 types: adjacent proximal surfaces ('single' or 'double' attacks); mesial and distal surfaces of the same tooth ('unilateral' or 'bilateral' attacks), and corresponding sites on the right and left sides of the mouth ('asymmetrical' or 'symmetrical' attacks). The general findings indicate that the ratio of individuals with single/double, unilateral/bilateral or asymmetrical/symmetrical attacks tends to fall to a limiting value with age. The limiting value, which in all cases is greater than zero, is reached at different levels and at different ages according to which pair of sites is involved, but once achieved remains essentially stable throughout the rest of life.

The fact that these ratios never reach zero means that there are always some individuals in whom only one member of the pair of sites being studied is decayed or filled, while the other is sound. In other words, sites that might be expected to succumb to caries because the adjacent surface, another site on the same tooth or the corresponding contra-lateral site has succumbed, never do. This has been interpreted as evidence for two qualitatively different kinds of site, caries vulnerable and caries resistant.

The theory of caries aetiology that has been developed from these observations involves two fundamental propositions: first, that superimposed on a general susceptibility to caries is a pattern of relatively susceptible and resistant sites within each mouth that depends on the genotype of the individual; and second, that the particular vulnerability of specific sites arises through 'auto-aggressive' attacks on distinct sets of odontoblasts, rendering the associated hard tissue susceptible to the caries process.

The purpose of this article is to summarise the theory and the observations and arguments used in its support, to comment on the plausibility of the theory, and to suggest a simple alternative hypothesis, in keeping with the current majority view of caries aetiology, to explain the failure of the single/double, unilateral/bilateral and asymmetrical/symmetrical ratios to reach zero.

Jackson's Theory

The Proposed Developmental Basis for Different Patterns of Site Attack

The existence of an inherited susceptibility to caries at particular anatomical sites, different patterns of susceptibility being associated with different genotypes, is supported by greater concordance for particular patterns of site attack between identical as opposed to fraternal twins[14]. The mechanism suggested to account for the inheritance of different patterns of site attack involves 3 main assumptions: first, that there are multiple qualitatively distinct sets of odontoblasts within each tooth; second, that the distribution of these sets is under genetic control, so that the potential exists for a difference in pattern of distribution between all individuals except identical twins; and third, that the pattern of caries susceptibility at the enamel surface is related to the way in which these specificities have been distributed within the odontoblast layer during development.

It has been suggested that the distinctness of these proposed odontoblast sets occurs through differences in the type, siting and orientation of antigenic determinants on the cell membrane[15], and that the pattern of distribution of different sets plays some part in determining tooth shape. The following are quotations from the original papers:

> In our view, the complicated morphology of a given tooth, and the difference, for example, between right and left homologous teeth, derive in part from multiple, distinctive sets of odontoblasts, together with the architecture of their associated protein fibres in dentine and enamel[5].
>
> We hold, for example, that the odontoblasts within a given tooth comprise a vastly complicated mosaic of distinctive elements. The complex shapes of organs (including teeth) derive, we propose, from the multiplicity of mosaic elements, the contact relations between them, and the morphology of individual cells and extracellular processes. We conclude that cells with a particular TCF [tissue coding factor] are distributed at one or more anatomical sites and that, in general, the distribution differs from one genotype to another. Morphological differences between genotypically-dissimilar individuals can be accounted for in this way[5].

*University of Edinburgh, Department of Oral Medicine and Oral Pathology, Old Surgeons Hall, High School Yards, Edinburgh EH1 1NR, and University Department of Human Genetics, Western General Hospital, Edinburgh EH4 2XU.

Comments

A significantly greater concordance for pattern of site attack between identical as opposed to fraternal twins does not imply a pattern of caries vulnerability or resistance intrinsic to the dental hard tissues. The greater concordance shown by identical twins could presumably be imposed by a variety of inherited characteristics such as tooth morphology, tooth position and salivary constitution, as well as more indirectly (because identical twins are more alike with respect to taste preferences, temperament and ability) through such environmental factors as diet and oral hygiene practices.

The existence of multiple sets of antigenically distinct cells of the same histological type, and the control of morphogenesis through the relative positions of these different cells, is not a concept favoured by modern developmental biologists. Current thinking, based on observation, experiment and computer simulation, is that the development of form and pattern is usually much simpler than it appears at first sight. A relatively small number of simple cellular forces can give rise to complex changes of form, regional differentiation generally being compatible with control by quantitative gradients rather than by a mosaic of qualitatively distinct elements[17–20].

The Autoaggressive Hypothesis

The hypothesis of autoaggressive disease proposed by Burch[21] assumes that normal growth is controlled by humorally distributed mitotic control proteins (MCPs) synthesised by the lymphoid system. Each antigenically specific cell type in the developing body carries a unique tissue coding factor (TCF) and the growth of each cell type is controlled by the correspondingly specific MCP. Somatic mutation in a lymphoid stem cell can produce a 'forbidden clone' which synthesises a mutant form of MCP which, rather than promoting growth, is pathogenic towards the cell type bearing the complementary TCF, resulting in tissue destruction and the initiation of an autoaggressive disease:

> We have proposed that autoaggressive disease is initiated when appropriate genes, in one or more central cells synthesising distinctive MCPs, become changed through a spontaneous somatic mutational process. The target cells suffering an autoaggressive attack bear TCFs complementary to a mutant and pathogenic MCP. These are the target cells whose normal growth is regulated by the MCP in its non-mutant form. Hence, if this theory is essentially correct, the systematics of the site distribution of the lesions of an autoaggressive disease should reflect the systematics of morphogenesis'.

It is argued that the age distribution of dental caries at each anatomical site is consistent with initiation of the disease process by one or more specific and independent random events followed by a latent period before clinical caries is detected. It has been suggested that these random events are somatic mutations, and that dental caries can be explained by the hypothesis of autoaggressive disease:

> In theory, the age-prevalence of dental caries at a particular site, or set of genetically associated sites, depends on: (i) the proportion of the population that is genetically-predisposed, (ii) the number of pathogenic forbidden clones, (iii) the average rate of their initiation (iv) the average interval, or latent period, between the initiation of the last forbidden clone and th. emergence of dental caries. It follows that differences in the age-prevalence of dental caries between any two sites might in theory arise from any one or more of the factors (i)-(iv)'.

The general expression that has been proposed for the age specific prevalence, P, of caries at a given site is

$$P = F_0 (1 - e^{-k^n})^t$$

where F is the percentage of the population at risk, the average rate of each type of initiating event (somatic mutation) is k per individual per year, initiation of the disease process occurs at age t by the accumulation of n somatic mutational events, but where the clinical manifestation of disease is delayed for λ years (the latent period) so that clinical onset is observed at age $t + \lambda$ years[18].

The assumption is that the target cells are odontoblasts, and that once they have suffered an autoaggressive attack, their associated hard tissue is rendered susceptible to the diffusion of exogenous cariogenic factors:

> We suggest that damaged odontoblasts probably fail to maintain the integrity of the associated protein matrix in dentine and enamel. In this way, perhaps, the denuded crystallites of the tooth are rendered vulnerable to the action of exogenous factors such as acids, and possibly, chelating agents'.
>
> In one person, certain sets of odontoblasts may be at risk with respect to an autoaggressive attack, but in another person, of dissimilar genotype, different sets of cells may be at risk'.

Comments

In order to accommodate the change in caries prevalence with age shown by different sites it is necessary to postulate different proportions of the population genetically predisposed, different mutation rates, different numbers of forbidden clones required for the initiation of disease, and different latent periods [illegible]. The theory therefore rests on the conformity of age prevalence observations with a mathematical model in which there are 4 independent variables, any one or combination of which can be arbitrarily manipulated to produce a fit with a new set of data. Manipulation of these 4 variables can produce a very wide range of expectations indeed, so that the fit of observations with the model is very unrigorous evidence for the hypothesis.

No clear explanation has been given as to how the resistance of surface enamel to caries can be influenced by the status of underlying odontoblasts. If odontoblast integrity were the criterion for caries resistance, it might be expected that teeth rendered non-vital by trauma should be more susceptible to caries than comparable teeth in control subjects.

The concept of particular sets of odontoblasts 'at risk' with respect to an autoaggressive attack seems to imply either that only certain MCPs are susceptible to mutation while others are not, or that only certain TCFs are vulnerable to corresponding mutant MCPs, and that these differences vary with the genotype of the individual. This introduces a further level of complexity into the hypothesis, for no apparent reason other than that it is required to provide expectations consistent with the observations.

Additional Observations and Arguments Used in Support of Jackson's Theory

The relationship between lingual pit caries and proximal

caries of the maxillary incisors. Caries prevalence for permanent molars.

Among individuals with some kind of caries experience in maxillary incisors the proportion of decayed or filled (DF) proximal sites in subjects with DF lingual pits was about half that in subjects with caries-free or unfilled pits[1]. Furthermore, maxillary incisors showing both DF lingual pit and proximal sites had an appreciably higher proportion of bilateral attacks than incisors without DF pits[2]. These findings were taken as evidence for a genetic difference between individuals with and without lingual pit caries.

In contrast to observations in most Western countries, caries prevalence for the second permanent molars of a Nigerian sample has been found to be approximately twice as high as for first permanent molars. This finding has been interpreted in terms of a genetic difference between Western populations and the Nigerian sample[11].

Comment

The existence of two different classes of individual, either within the same population or in two different populations, although consistent with a genetic difference between them, cannot be regarded as evidence for such a difference. If this were the case it could just as well be said that individuals who visit the dentist regularly are likely to be genetically different from those who do not, or that those who have acrylic partial dentures are genetically different from those who have chrome-cobalt partial dentures, and so on. The only way to demonstrate a genetic basis for the observed difference would be to study the distribution of individuals with these particular patterns of site attack in families. Even if such a study supported a genetic interpretation, this would still not imply that the difference was mediated through intrinsic vulnerability of the dental hard tissues.

The stability of caries pattern prevalence with time and place

The consistency of caries pattern prevalence from year to year and in different parts of the country has been taken as evidence supporting the proposed genetic control of specific site vulnerability:

> In the years 1959, 1960 and 1961 the percentage of individuals with caries experience (ie DMF teeth) in maxillary incisors was 49 per cent, 48 per cent and 49 per cent respectively. The remarkable regularity of these values obtained from individuals from all parts of the UK strongly suggests a community characteristic which has a genetic basis[1].

Comment

The lack of change in frequency of a particular attribute in a population with time, or the uniformity of its frequency in populations from different areas, provides no evidence for genetic control of the attribute. Such stability of frequency could equally well apply to completely non-biological characteristics. Population data cannot provide information about the way in which a characteristic is transmitted from one generation to the next. The study of inheritance clearly demands the use of related individuals.

Symmetry of site attack within individuals

Even though the asymmetrical/symmetrical attack ratios do not fall to zero with age, the degree of symmetry of attack is still considerably greater than would occur if each site were attacked independently of the corresponding contra-lateral site. This symmetry has been observed in both maxillary and mandibular incisors, and it has been suggested that the findings implicate an endogenous contribution to caries[2, 10]. Other symmetrical patterns of site attack on the maxillary incisors also appear to occur more frequently than would be expected by chance[4, 7].

The average number of DF maxillary incisor proximal sites per person increases slightly from 15 to 30 years, but the relative prevalence of individuals with 1, 2, . . ., 8 DF sites remains fairly stable with age. There is a tendency for similar levels of prevalence for 1 and 2, 3 and 4, 5 and 6, and 7 and 8 sites attacked. This non-random pattern has been taken as evidence for genetically controlled site vulnerability[4].

Comments

If correlation for caries attack between corresponding sites on the two sides of the mouth were complete, only even numbers of DF sites would be found. If there were no correlation, and attack occurred at each site independently of the others, the prevalence of 1, 2, . . ., 8 DF sites would fall off in a graded fashion. The intermediate situation observed suggests a degree of positive correlation and therefore of symmetry, which is consistent with the more direct observations of symmetry of site attack.

If different TCFs are responsible for the morphology of corresponding sites on the two sides of the mouth[2, 21], and if mutation for MCPs is at random[4, 7], attacks at corresponding sites on the two sides should be uncorrelated. This theoretical expectation is not consistent with the degree of symmetry of attack observed. Correlated attacks on the two sides of the mouth can however be explained in terms of common constitutional and local environmental factors.

Prevalence of mirror image patterns of site attack

The prevalence of a particular pattern of site attack is generally similar to that of the corresponding mirror image pattern[2, 3]:

> Another intriguing feature of these data is the prevalence of mirror image patterns. The pattern (d)-S occurs with the same order of frequency as does S-(d); (dm)-(d) has the same order of frequency as (d)-(dm) and so on. These patterns are in complete accord with the genetic hypothesis and in no way can they be explained on the basis of the acid theory or any other environmental theory[4].

Comment

The genetic hypothesis proposes that corresponding sites on the right and left sides of the mouth are specified by different TCFs, and that each TCF is coded for by a particular genotype. Similar prevalence of mirror image patterns therefore requires similar population frequencies for 'right sided genes' and 'left sided genes.' It would add considerable complexity to the theory to explain how and why these similar frequencies are maintained. On the other hand, the correlation for site attack observed between sides within individuals, which can be explained simply in constitutional and local environmental terms, would automatically result in a degree of similarity of prevalence

for mirror image patterns.

Cervical caries on maxillary incisors

It is argued that, according to the acid theory, because plaque is usually distributed symmetrically on the labial surfaces of maxillary incisors, cervical caries should also be distributed symmetrically. Considering only those individuals who showed some cervical caries on either the permanent maxillary central or lateral incisors, the percentage ratios 'right side DF: both sides DF: left side DF' were found to be 33:33:33 for central incisors and 40:20:40 for lateral incisors. The presence of a substantial proportion of individuals with only one side DF, despite the presumed symmetrical distribution of plaque, was taken as evidence for genetically controlled site specific vulnerability to caries[9].

Comment

Although a substantial proportion of individuals was found to have asymmetrical attacks, the ratios are compatible with a degree of positive association between sides. Suppose for example that 10 per cent of the population had some cervical caries on maxillary lateral incisors. The population frequency of right side lesions, which is the same as that of the left side lesions, would be $10\% \times (40\% + 20\%) = 0{\cdot}06$. If attack on the right side were independent of attack on the left, the expected frequency of symmetrical attacks would be $0{\cdot}06^2 = 0{\cdot}0036$. However, with a 10 per cent prevalence of cervical caries in the population, the observed proportion of individuals with symmetrical attacks would be $10\% \times 20\% = 0{\cdot}02$, which is approximately 6 times greater than expected if there were no correlation between sides. The observations therefore do suggest some degree of symmetry of attack, which is not consistent with independent genetic control of attack at corresponding sites on the two sides of the mouth.

The proportion of caries-free sites in the dentition as a whole

[It has been shown] that a substantial proportion of approximal sites remains caries-free throughout the lifespan and would remain caries-free even on the assumption that all approximal sites on missing teeth were, or would have become, carious. It is concluded that the acid theory can offer no convincing explanation for these and previously published data without invoking an essential contribution from intrinsic host factors, specific to particular sites on teeth[10].

Comment

There is considerable evidence to indicate a graded increase of enamel resistance with increasing exposure to the oral environment. This implies that the longer a site remains sound the less likely it is to suffer a carious attack. Initial small quantitative differences of vulnerability or local environment between sites in the same mouth could therefore be amplified with age, resulting in the apparently qualitative all-or-none phenomenon of vulnerability as opposed to resistance. Some of the evidence for, and consequences of, this graded increase in resistance with age are considered below.

Invoking the Hardy-Weinberg law

The Hardy-Weinberg law, a fundamental theorem of population genetics, has been invoked in an attempt to support the autoaggressive explanation of caries aetiology. This law states that for an autosomal locus at which there can be either allele A (at frequency p) or allele a (at frequency q = 1-p), if the population is infinitely large, if mating within it is at random, and with certain other reservations, the 3 genotypes AA, Aa and aa will appear in the proportions p^2, 2pq and q^2 respectively.

Burch[21] has arbitrarily assigned hypothetical genotypes to different caries phenotypes, arbitrarily chosen the gene frequency, and then drawn attention to the similarity between the observed frequencies of these chosen phenotypes in the population and those expected on the basis of the Hardy-Weinberg law.

Comment

This procedure is entirely without justification and provides no support for Jackson's theory. The Hardy-Weinberg law can only be used to provide support for a genetic hypothesis when applied to family data. If population data alone are available, the law must be applied to a known locus, previously identified through a study of related individuals, when its purpose is to test whether or not the population is in genetic equilibrium[22].

Anomalous Observations

The asymmetrical/symmetrical attack ratios for maxillary incisor lingual pits

In contrast with the behaviour of the asymmetrical/symmetrical attack ratios for proximal sites on the maxillary incisors, the ratio for lingual pit sites on the maxillary lateral incisors increases with age until at least 40 years, after which it remains relatively stable. For lingual pit sites on the maxillary central incisors the ratio shows a more complex age dependence, with an initial rise from 15 to 19 years followed by a fall to about 49 years, with relative stability thereafter. Distortion of the natural pattern of age dependence for this ratio through symmetrical placement of preventive restorations in the younger age groups has been suggested as a factor contributing to this anomalous finding[7].

The single/double attack ratios for mandibular incisors

In contrast to the behaviour of the single/double attack ratios for maxillary incisors, the corresponding ratios for mandibular incisors increase with age:

The change of ratio [for mandibular incisors] with age indicates: (i) that a higher proportion of the examined population is genetically-predisposed to single- than double-attacks, and either (ii) that the average rate of initiation of forbidden clones that result in double-attacks is higher than for those that result in single attacks, or (iii) that the average latent period between the initiation of the last pathogenic forbidden clone and the first appearance of clinical dental caries is shorter for double- than for single-attacks, or (iv) that both (ii) and (iii) are effective[12].

Comment

The interpretation of the difference in age dependence of the single/double attack ratios between maxillary and mandibular incisors in terms of the autoaggressive hypothesis requires an additional assumption, namely that the frequency with which common TCFs occur in association with adjacent sites in the mandible is different from the frequency with which they occur in the maxilla.

Since the morphology of maxillary and mandibular incisors is basically the same, it is difficult to see why this should be the case. On the other hand, it is known that the local environment of the two groups of incisors is very different.

An Alternative Hypothesis

It is well known that the proportion of all sites carious tends to level off after a relatively rapid initial increase with age. This reduction in the rate of initiation of new carious lesions has been interpreted by Jackson as being due to the saturation of intrinsically vulnerable sites by caries, leaving sound those sites that are intrinsically resistant. Although this explanation has been applied to a vast array of site attack observations, there is no convincing evidence from any other independent source to support it.

There is, however, a variety of compelling evidence to show that changes occur in the surface enamel with exposure to the oral environment. These changes have been summarised by Jenkins[23] who cites a number of original references. 4 relevant points will be mentioned here: first, several investigations have shown that the permeability of enamel falls with age; second, the enamel surface concentration of fluoride increases after eruption, the areas of fluoride uptake being particularly or perhaps exclusively those that have suffered early demineralisation; third, the solubility of surface enamel in acid decreases with age; and fourth, if two comparable groups of rats are fed a cariogenic diet, one immediately after weaning and the other some weeks later, more caries develops over a given period in the first group than in the second. These findings imply an overall graded increase in resistance of the enamel to caries with age. The observed change in prevalence of attack with age at a given site (fig. 1) can be interpreted as a direct consequence of this graded increase in resistance.

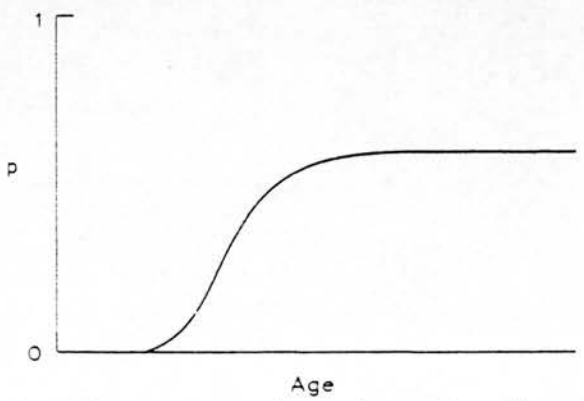


Fig. 1.—The general pattern of observed change in prevalence of attack, p, at a given site with age (after Jackson[1]).

A Quantitative Model

Given only the age specific prevalence of attack for each member of a pair of sites it is possible to use a simple quantitative model to predict change in the single/double, unilateral/bilateral or asymmetrical/symmetrical ratios with age, assuming different levels of association of attack between the two sites within individuals, mediated through common constitutional and local environmental factors. The relationship between the prevalence of attack at site 1 (p), the prevalence of attack at site 2 (q) and the proportions of the four different combinations of attack at the two sites (a,b,c,d,) can be summarised as shown in Table I, where

$a + b = 1 - q, \quad c + d = q, \quad a + c = 1 - p,$
$b + d = p, \quad a + b + c + d = 1.$

TABLE I.—THE PROPORTIONS OF INDIVIDUALS (a, b, c, d) SHOWING DIFFERENT COMBINATIONS OF ATTACK AT A PAIR OF SITES. THE PREVALENCE OF ATTACK AT SITES 1 AND 2 ARE p AND q RESPECTIVELY

		Site 1		
		Sound	*Attacked*	
Site 2	*Sound*	a	b	1-q
	Attacked	c	d	q
		1-p	p	1

When carious attacks at a pair of sites are independent or uncorrelated the relationship ad = bc will hold for large samples. This implies that the proportion of individuals with attack at site 2 is the same for those with and without attack at site 1 (d/b = c/a). Any association between attack at the two sites will result in an increase in a and d at the expense of b and c. There are several measures that can be used to describe the degree of association in such a situation, including various versions of the correlation coefficient[24]. The measure chosen here is the 'cross-product ratio', 'odds ratio' or 'relative risk'[25]. It has the advantage of a simple epidemiological interpretation at different levels of prevalence.

The cross-product ratio can be defined in terms of Table I as the odds of attack at site 2 when site 1 is attacked (d/b) relative to the odds of attack at site 2 when site 1 is sound (c/a). The relative risk, α, is therefore given as

$$\alpha = \frac{d/b}{c/a} = ad/bc$$

When attacks at the two sites are independent, $\alpha = 1$. The greater the relative risk the higher the proportion of double attacks.

Using the relationships in Table I:

$$d = \frac{\alpha bc}{a} = \frac{\alpha(p-d)(q-d)}{1-p-q+d}$$

Therefore

$$d^2 + d(1-p-q) = \alpha[pq - d(p+q) + d^2]$$

so that

$$d^2(1-\alpha) + d[1-(p+q)(1-\alpha)] - \alpha pq = 0$$

and

$$d = \frac{-B + \sqrt{B^2 + 4AC}}{2A}$$

where

$$A = 1-\alpha$$
$$B = 1-(p+q)(1-\alpha)$$
$$C = \alpha pq$$

(The alternative solution for d with the square root negative is inadmissible.)
Since:

$$a = 1 - p - q + d$$
$$b = p - d$$
and $$c = q - d$$

it is possible to derive the expected proportions a,b,c,d, and thus the single/double ratio of $(b+c)/d$, for any chosen value of α, given only p and q. In other words, the model assumes that each pair of sites has its own odds ratio which remains constant as prevalence increases with age.

What are needed to test this model are attack prevalence figures for different ages and for each site independently, preferably the figures that apply to Jackson's own study populations in which the single/double, unilateral/bilateral and asymmetrical/symmetrical ratios were observed. However, Jackson's publications are concerned primarily with individuals who show attacks rather than the proportion of the population that these individuals represent. Nevertheless, an average age specific prevalence, p, for all incisor proximal sites can be derived from data given by Jackson, Burch and Fairpo[14]. These average figures (Table II) are adequate to show how the model can be applied.

TABLE II.—AVERAGE AGE SPECIFIC PREVALENCE OF CARIES ATTACK, p, OVER ALL INCISOR PROXIMAL SITES[14]

Age (years)	*Total sites*	*DF sites*	*p*
15-19	763,060	52,170	0·068
20-24	649,478	56,282	0·087
25-29	486,984	45,881	0·094
30-34	392,040	40,929	0·104
35-39	300,746	32,432	0·108
40-44	233,782	25,118	0·107
45-49	158,676	17,339	0·109
50-54	98,586	10,981	0·111
55-59	54,736	6,095	0·111

The predicted single/double ratios derived from the p values listed in Table II are illustrated for different values of α in figure 2, assuming the same prevalence of attack for each member of the hypothetical pair of sites ($p = q$). The pattern of change of the ratio with age is the same as that observed by Jackson (with the exception of the anomalous findings discussed earlier) and shows that the stabilised value occurs at a progressively lower level with increasing relative risk, as would be expected. Furthermore, the higher the relative risk, the earlier the stabilised value is reached. Curves for $\alpha = 1$ and $\alpha = \infty$ correspond to a correlation between sites of $r = 0$ and $r = 1$ respectively. The simple quantitative model described can therefore generate, directly from single site attack prevalence data,

TABLE III.—JACKSON'S OWN OBSERVED STABILISED RATIOS FOR 4 DIFFERENT PAIRS OF SITES[14] TOGETHER WITH A SUMMARY OF WHETHER THE SITES INVOLVED HAD MIRROR IMAGE SURFACES AND/OR A COMMON CONTACT POINT

Sites	*Stabilised Jackson ratio*	*Mirror image surfaces*	*Common contact*	*Estimated α*
RCe(m)—LCe(m)	0·7-0·8	–	–	76
Ce(d)—La(m) (R or L)	1·1	–	–	39
RCe(d)—LCe(d)	1·4	–	–	27
Ce(m)—Ce(d) (R or L)	2·5	–	–	11

R = right, L = left, Ce and La = maxillary central and lateral incisors, m = mesial, d = distal. The approximate estimate of the relative risk, α, was calculated by assuming a prevalence of attack of 11 per cent for each site when the stabilised ratio had been achieved (see Table II, older age groups).

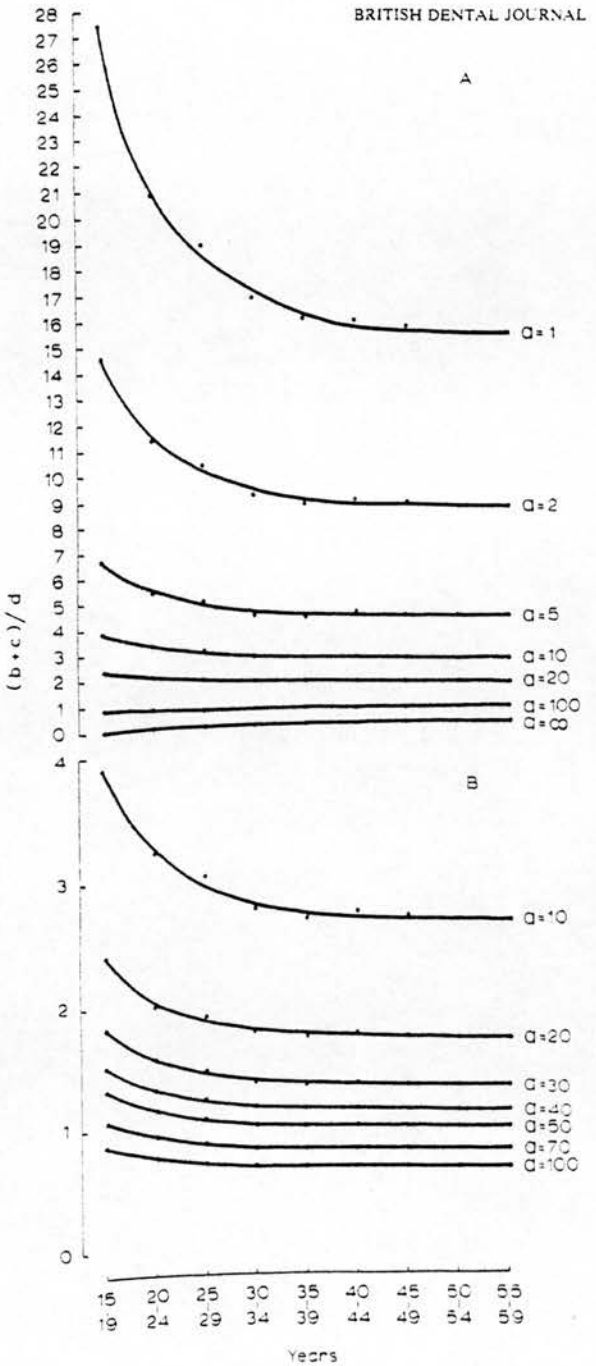


Fig. 2.—Predicted single/double ratios, $(b+c)/d$, for a hypothetical pair of incisor sites, derived from p values listed in Table II for different values of α with $p = q$. Plot A shows a full range of curves. Plot B shows curves covering a range of stabilised ratios comparable to that observed by Jackson.

ratios comparable to those observed. This can be done by incorporating only a single variable, the level of association for carious attack between sites, expressed in terms of relative risk.

In the light of the model, the levels at which Jackson's observed single/double, unilateral/bilateral and asymmetrical/symmetrical ratios stabilise are of some interest in that they imply different levels of relative risk for different pairs of sites. Table III lists the observed

stabilised ratios for 4 pairs of sites[11], along with approximate estimates of relative risk. The pair of sites with the highest relative risk had mirror image surfaces and a common contact point, the two pairs with intermediate relative risks had either a common contact point or mirror image surfaces but not both, and the pair of sites with the lowest relative risk had neither mirror image surfaces nor a common contact point. These findings are consistent with an important local environmental contribution to carious attack, and therefore with the current majority view of the aetiology of dental caries.

Conclusions

Jackson's theory of caries aetiology is a complex one requiring the co-ordinated operation of a number of improbable mechanisms for which no independent supporting evidence is available. The reduction in incidence of new carious cavities with age, and in particular the stabilising of Jackson's single/double, unilateral/bilateral and asymmetrical/symmetrical ratios at levels above zero, can be plausibly explained in terms of a simple quantitative model that assumes a graded increase in enamel resistance with exposure to the oral environment (for which there is considerable independent evidence), together with a variable degree of association for carious attack between sites within individuals, mediated through common constitutional and local environmental factors.

ACKNOWLEDGMENT

The author is grateful to Gillian Raab, University of Edinburgh Medical Computing and Statistics Unit, for statistical advice.

REFERENCES

[1] Jackson D. Genes and dental caries. *Proc Roy Soc Med* 1968; **61**: 265–269.
[2] Jackson D. Genetic endowment and dental caries. *Arch Oral Biol* 1971; **16**: 1433–1441.
[3] Jackson D, Burch P R J. The anatomical site distribution of clinical dental caries in the maxillary incisor teeth of 11- and 12-year-old children. *Arch Oral Biol* 1968; **13**: 809–817.
[4] Jackson D, Burch P R J. Dental caries: distribution, by age-group, between homologous (right-left) mesial and distal surfaces of human permanent maxillary incisors. *Arch Oral Biol* 1970; **15**: 1059–1067.
[5] Jackson D, Burch P R J. Non-random distributions of caries attacks among mesial and distal surfaces of human permanent maxillary incisors. *Arch Oral Biol* 1972; **17**: 119–125.
[6] Jackson D, Fairpo C G, Burch P R J. Caries-resistant sites on human maxillary incisor teeth. *Arch Oral Biol* 1972; **17**: 495–501.
[7] Jackson D, Fairpo C G, Burch P R J. Human lingual pit caries: distribution between right and left maxillary incisors. *Arch Oral Biol* 1973; **18**: 181–187.
[8] Jackson D, Fairpo C G, Burch P R J. Distribution of symmetric and asymmetric patterns of caries attack in human permanent maxillary incisor teeth: genetic implications. *Arch Oral Biol* 1973; **18**: 189–195.
[9] Jackson D. Sugar and dental caries. Reprinted from the *Probe*. London: Bouverie Pub Co Ltd, 1978.
[10] Jackson D, Sutcliffe P, Burch P R J. The anatomical site distribution of clinical dental caries in the mandibular incisor teeth of 11- and 12-year-old children: aetiological implications. *Arch Oral Biol* 1967; **12**: 1343–1353.
[11] Jackson D, Burch P R J, Fairpo C G. Dental caries: distribution, by age-group, between the mesial and distal surfaces of human permanent mandibular incisors. *Arch Oral Biol* 1972; **17**: 1343–1350.
[12] Jackson D, Burch P R J, Fairpo C G. The distribution of clinical dental caries between the adjacent surfaces of neighbouring mandibular incisors. *Arch Oral Biol* 1972; **17**: 1351–1355.
[13] Akpata E S, Jackson D. Caries vulnerability of first and second permanent molars in urban Nigerians. *Arch Oral Biol* 1978; **23**: 795–800.
[14] Jackson D, Burch P R J, Fairpo C G. Caries-free sites on teeth: aetiological implications. *Br Dent J* 1979; **147**: 63–66.
[15] Fairpo C G. Comparison of dental caries experience in identical and like-sexed fraternal twins. *J Dent Res* 1968; **47**: 971.
[16] Burch P R J, Jackson D. Periodontal disease and dental caries. Some new aetiological considerations. *Br Dent J* 1966; **120**: 127–134.
[17] Wolpert L. Positional information and pattern formation. *Curr Top Dev Biol* 1971; **6**: 183–224.
[18] French V, Bryant P J, Bryant S V. Pattern regulation in epimorphic fields. *Science* 1976; **193**: 969–980.
[19] Gordon R, Jacobson A G. The shaping of tissues in embryos. *Sc Amer* 1978; **238**: 80–87.
[20] Williams G J A, Caveney S. A gradient of morphogenetic information involved in muscle patterning. *J Embryol Exp Morph* 1980; **58**: 35–61.
[21] Burch P R J. *An inquiry concerning growth, disease and ageing*. Edinburgh: Oliver and Boyd, 1968.
[22] Sofaer J A. Dental morphologic variation and the Hardy-Weinberg law. *J Dent Res* 1970; **49**: 1505–1508.
[23] Jenkins G N. *The physiology and biochemistry of the mouth*. Oxford: Blackwell, 1978.
[24] Blalock H M. *Social statistics*, p 225–239. New York: McGraw-Hill, 1960.
[25] Armitage P. *Statistical methods in medical research*, p 426–428. Oxford: Blackwell, 1971.

REVIEW ARTICLES AND CONTRIBUTIONS TO BOOKS

This section includes contributions to a review of current dental research, undergraduate and postgraduate dental texts, and a major new medical genetics text.

Reprinted from British Medical Bulletin, *1975, Vol. 31, No. 2* (Research in Dentistry), *pages 107–110*

GENETIC VARIATION AND TOOTH DEVELOPMENT

J. A. SOFAER B.D.S. Ph.D.

School of Dental Surgery
and
Department of Human Genetics
University of Edinburgh

Teeth arise from the dental lamina, a band of thickening of the oral epithelium that marks out the position of each future dental arch. The lamina forms as an ingrowth of oral epithelial cells into the underlying mesenchyme of the jaw, and from it, by further cell proliferation, develop localized swellings that go on to form tooth germs. Each bud of lamina destined to be a tooth germ becomes invaginated, producing a bell-shaped epithelial structure that increases in size and morphological complexity, until the basic configuration of the future tooth crown is fixed with the onset of dentine and enamel matrix apposition and mineralization. Enamel formation usually ceases once the tooth crown is complete, but dentine formation continues with root development. A layer of cementum is laid down on the outer surface of the root dentine and incorporates periodontal fibres that support the tooth through their attachment to the bony wall of its socket. Genetic variation may affect each of these stages of tooth development.

1. Initiation

Variation in the capacity to initiate tooth germs results in some individuals possessing more, and others less, than the normal complement of teeth. Studies of man and experimental animals show that both failure of initiation and the capacity to initiate additional teeth may be at least partly inherited.

The mouse normally has three molars on each side of each jaw, but the third molar, which is usually initiated soon after birth, sometimes fails to develop. Failure to develop may be a characteristic of a particular wild population or laboratory strain, or one aspect of a syndrome of abnormalities produced by a single mutant gene. For example, some years ago the inbred strains CBA, A and C57BL were found to have, respectively, 17.9%, 2.3% and 0.1% of individuals with one or more missing third molars, and crosses between strains confirmed the genetic basis for this difference between them (Grüneberg, 1963). In the mouse mutant *crooked*, which develops anomalies of the axial skeleton, and the mutant *tabby*, which develops a generalized abnormality of epidermal derivatives, third molars may again be absent (Grüneberg, 1965); though, strangely enough, *tabby* mice may possess an additional molar anterior to the first molar of the normal series. Supernumerary molars have also been found in a strain of rice rat, possibly related to a single gene (Sofaer & Shaw, 1971); and additional incisors in the mouse (Danforth, 1958) and in the dog (Hitchin & Morris, 1966) may have a hereditary basis.

Embryological study of the third molars of CBA and *crooked* mice (Grewal, 1962), and of the supernumerary and third molars of *tabby* mice (Sofaer, 1969b), indicates that the critical stage for initiation of a tooth germ is after localized laminal proliferation has started but before invagination takes place. Localized proliferations from the dental lamina that normally go on to form established tooth germs can therefore sometimes regress.

The initiation of a tooth germ depends on interaction between the proliferating dental epithelium and its underlying mesenchyme. Both tissues must function normally if a normal tooth germ is to be formed. In the mouse mutant *tabby*, and in another mutant, *downless*, which is phenotypically indistinguishable from *tabby*, there is, in addition to the abnormalities of tooth germ initiation, a virtually complete failure of initiation of hair follicles in the tail. Hair follicle development also depends on epithelium–mesenchyme interaction, and, in its early stages, is very similar to that of tooth germs. The suppression of tail hairs in these mutants can therefore be used to investigate how the abnormalities of initiation arise. Failure of initiation could be due to failure in either the epidermal (epithelial) or the dermal (mesenchymal) component of the system, or both. Reciprocal recombinations between *downless* homozygote (phenotypically mutant) and heterozygote (phenotypically normal) tail epidermis and dermis have been made prior to the time when the first signs of follicle formation are visible in the tails of normal mice, and the recombined elements allowed to continue growth and differentiation in culture. Explants composed of heterozygote epidermis with either heterozygote or homozygote dermis produced follicles, whereas explants composed of homozygote epidermis with either homozygote or heterozygote dermis did not. Failure of initiation in *downless* therefore appears to result from an abnormality restricted to the epidermis (Sofaer, 1973a). However, similar experiments with *tabby* provided no evidence of a primary epidermal effect (Sofaer, 1974), suggesting that, although the two genes produce the same development result, they may do so in different ways.

The most commonly missing tooth in most human populations is also the third molar, the incidence of individuals with one or more missing third molars varying from around 1% in some African Negro and Australian aboriginal samples to estimates of over 30% among Japanese, with values for Caucasians lying somewhere between. For teeth other than third molars, six large studies of different populations have shown the incidence of individuals with missing teeth to vary from 2.3% to 9.6% (Pindborg, 1970); and these different frequencies probably, though not necessarily, reflect a degree of hereditary determination for the absence of teeth. Apart from the third molar, one of the most frequently missing teeth is the upper lateral incisor, absent in 1–3% of individuals of most samples examined, but in over 20% of residents of the isolated Swiss mountain village of Illgau (Jöhr, 1934). The high incidence in a small, isolated and therefore probably relatively inbred community suggests a genetic basis for the abnormality, and family studies have shown that the condition is indeed highly heritable, with the possibility of a single gene making a major

contribution (Mandeville, 1950; Alvesalo & Portin, 1969). However, a family study of missing teeth in general, excluding third molars, points to a largely quantitative genetic basis, since not only is the incidence of missing teeth higher than normal among relatives of those in whom teeth have failed to develop, but the greater the number of missing teeth in any individual the higher the incidence of individuals with missing teeth among his relatives (Grahnén, 1956).

In man, missing teeth and supernumerary teeth may also be part of generalized syndromes of abnormalities caused by single mutant genes (Gorlin & Pindborg, 1964). A striking example of such a circumstance is shown by individuals with hypohidrotic (or anhidrotic) ectodermal dysplasia, usually an X-linked recessive condition where many, and occasionally all, of the teeth fail to develop. This is a general abnormality of ectodermal derivatives and it is therefore not difficult to understand how the teeth may be involved; but for some syndromes the developmental relationship between missing or supernumerary teeth and the other abnormalities may not be so clear. For instance, in cleidocranial dysostosis, an autosomal dominant condition that includes absence or hypoplasia of the clavicles and abnormal ossification of the skull, numerous though frequently unerupted supernumerary teeth may occur, despite the fact that teeth of the normal series sometimes fail to develop.

2. Growth

The genetic control of the growth of tooth germs acts through a number of different influences. Firstly there is the intrinsic potential of the tooth germ itself, controlled by genes that affect directly the rate of proliferation of dental epithelium and its associated mesenchyme; secondly there is the immediate local environment around the developing germ, influenced by genes affecting the tooth germ's nutritional requirements through, for example, blood supply and the number, size and proximity of competing contemporary tooth germs; and thirdly there is the general environment of the individual as a whole, contributed to in the early stages by the lactational performance of the mother. In experimental situations it may be possible to study these influences separately. For example, the inbred mouse strain A has small lower third molars, and small size can be attributed to the genotype of the A strain; but if A strain young are suckled by more vigorous hybrid mothers their third molars grow a little larger. The growth of third molar germs therefore depends both on the genotype of the individual and on the genotype of his mother (Grüneberg, 1963).

In many cases, however, it is possible to study only the combined effect of all sources of genetic variation in producing size differences between adult individuals. In randomly bred populations of laboratory animals this combined effect can be expressed in terms of the total genetic variance, by comparing the observed variance of a chosen dental dimension with the corresponding variance in a genetically homogeneous laboratory sample, usually the hybrid of two inbred strains. The variance shown by hybrids is assumed to estimate the effect of all the non-genetic sources of variation, and when subtracted from the total randomly bred variance leaves the variance due to genetic differences between individuals in the randomly bred strain. This genetic variance expressed as a proportion of the total observed variance is known as the degree of genetic determination, and has been estimated at about 60% for the width of mouse lower first molars in four randomly bred laboratory stocks (Bader & Lehmann, 1965).

Another way to approach the problem is to study the resemblance between relatives, expressed either as a correlation or as an estimate of the heritability. Estimates of resemblance between relatives in both human and mouse populations indicate that relatives tend to be most alike with respect to the size of early developing teeth within a morphological class (incisors, premolars and molars), and least alike with respect to the last tooth to develop in each class. This means that the later a tooth develops in a given region of the jaw the more its size variation appears to depend on non-genetic differences and the less on genetic differences between individuals. This pattern of genetic variation can be interpreted in terms of compensatory interaction between tooth germs during development, the later developing teeth tending to compensate for the combined deviations of their earlier developing neighbours from the norm, and consequently showing signs of greater local environmental influence (Sofaer, 1973b).

In addition to considering different sources of genetic variation from a developmental point of view, it may also be possible to assess the relative contributions of sex-linked as opposed to autosomal genes in controlling tooth size. There are conflicting reports here. Some human investigations suggest that X-linked genes do contribute to the control of tooth size (Garn, Lewis & Kerewsky, 1965; Alvesalo, 1970), whereas others find no evidence of X-chromosome involvement (Niswander & Chung, 1965; Bowden & Goose, 1969). Since tooth size is dependent on many interacting factors, each individually subject to genetic control, it is likely that some genes involved are carried by the X chromosome. On the other hand, unless a sample contains detectable genetic heterogeneity at the X-linked loci concerned, there will be no evidence of X-linked control. In contrast to this is the finding that the teeth of males are consistently larger (by 2–6%) than those of females, an observation that could be explained by the difference of general physiology between the sexes, though a direct Y-linked effect has been suggested (Alvesalo, 1970).

Major genetically determined human conditions may also affect tooth size. A generalized reduction of tooth size has been reported in chromosomal aberrations—namely Down's syndrome and Turner's syndrome, as well as in conditions associated with single mutant genes—notably chondroectodermal dysplasia, focal dermal hypoplasia and Hurler's syndrome (Gorlin & Pindborg, 1964).

3. Morphodifferentiation

As tooth germs grow, morphodifferentiation of the crown proceeds in a regular sequence for each tooth, each cusp appearing in a prescribed developmental relationship to its neighbours. It is therefore not surprising to find situations where tooth size and shape are related. For example, cusp number and crown size of human lower first molars have been found to vary together (Garn, Dahlberg, Lewis & Kerewsky, 1966); and, in the *tabby* mouse, larger supernumeraries tend to be morphologically more complex (Sofaer, 1969c). However, it has been usual to consider dental morphological features as characters for independent study, and many observations show that they are under some degree of genetic control. Different minor morphological variants of mouse molar crowns are characteristic of different strains and are likely to depend on many genes for their expression (Grüneberg, 1965; Sofaer, 1969a); and gross dental morphological abnormalities in the mouse may be associated with single mutant genes (Grüneberg,

1965). In man, studies on twins have demonstrated a greater concordance of dental morphological characters within monozygotic pairs than within dizygotic pairs (Lundström, 1963).

An example of the kind of morphological variable that has been studied in man is the cusp of Carabelli, a feature that is sometimes present on the lingual surface of the mesiolingual cusp of some upper molars. In common with other dental morphological characters, the level of manifestation is variable and the incidence may differ from one population to another. Limited pedigree data have appeared in the past to support the contention that this character is controlled by a single gene (Kraus, 1951; Tsuji, 1958), but these are not sufficient to reach any firm conclusion about the inheritance of the feature; and, furthermore, there is direct evidence that the single-gene model does not apply, either to this or to certain other dental morphological characters (Lee & Goose, 1972). In fact, the patterns of variation of these features suggest that they are controlled by many genes and environmental factors, so that quantitative genetic methods rather than pedigree studies are probably more appropriate for their analysis. Such methods involve a study of the resemblance between relatives, and require family data that have not been available in sufficient quantity in the past.

In the absence of adequate family data it may be possible to infer something about the genetics of these characters by comparing their incidence in populations that are known to be genetically different. For instance, the cusp of Carabelli and shovel-shaped upper incisors appear to be good ethnic markers in Chilean populations with different proportions of Indian and Caucasian ancestry, the incidence of each character approaching that in each ancestral population with increasing contribution from that population (Pinto-Cisternas & Figueroa, 1968; Rothhammer, Benado & Pereira, 1971). More specifically, it may be possible to compare relationships between populations derived from a study of dental morphological variables with relationships based on gene frequencies for a number of established simple genetic polymorphisms. This can be done in the following way. Differences between populations in terms of allele frequencies at each polymorphic locus investigated (usually one controlling a blood-group or serum protein variant) can be combined over several loci to provide an estimate of genetic distance between populations. Similarly, differences between populations in terms of the frequencies of alternative forms of each dental morphological variant (for example, Carabelli's cusp either present or absent) can be combined over a number of variants to provide a tooth-based distance between populations. The relationship between three populations derived from genetic distance estimates, and the relationship between the same three populations derived from different tooth-based distances, have shown a degree of correspondence for some dental variants but not for others (Sofaer, Niswander, MacLean & Workman, 1972). Some dental morphological characters may therefore be good indicators of genetic difference between populations, and therefore largely under genetic control, whereas others may not.

Two examples of more gross morphological variants may also be of interest: fusion of two adjacent tooth germs during development, resulting in a composite double tooth; and abnormal invagination of the dental epithelium during morphodifferentiation, producing a dens in dente or dens invaginatus. In man, the incidence of each of these anomalies seems to vary from one racial group to another (Chung, Niswander, Runck, Bilben & Kau, 1972), and there are some human family data (Grahnén, Lindahl & Omnell, 1959; Brook & Winter, 1970) to suggest a substantial hereditary component in their aetiology.

Fusion of adjacent tooth germs is also known to occur in experimental animals. In the dog, incisor fusion, with or without an associated supernumerary incisor, has been found in a strain of Lakeland terriers, indicating at least a partly hereditary origin for the abnormality (Hitchin & Morris, 1966). In the rice rat, fusion may occur between the first and second molars, or even between all three molars of the normal series; and there is some evidence that this condition, which also may occur with or without an associated supernumerary tooth, is controlled by a single major gene (Sofaer & Shaw, 1971).

4. Matrix Apposition and Mineralization

There are two main processes involved in the development of human dental enamel: matrix apposition, when the basic tissue structure of the enamel is established; and mineralization, during which the mineral content rises to about 96%. There are correspondingly two broad categories of hereditary abnormalities of enamel formation: hereditary enamel hypoplasia (amelogenesis imperfecta of the hypoplastic type), primarily affecting matrix apposition; and hereditary enamel hypomineralization (amelogenesis imperfecta of the hypomineralized type), primarily affecting mineralization. The former results in a quantitative deficiency of more or less normally mineralized enamel, and the latter in a qualitative abnormality in enamel of more or less normal thickness. However, intermediate conditions where both qualitative and quantitative disturbances occur are relatively common, indicating that the two processes are not independent. Each of these categories of abnormality has been further subdivided according to details of the clinical presentation and histopathology, and the pattern of hereditary transmission (Witkop, 1965); but the majority of cases of genetically determined hypoplastic defects appear to be due to an X-linked gene, and the majority of those in the hypomineralized category to an autosomal dominant. An intriguing feature of the X-linked hypoplastic disturbance is the occurrence of "striped" teeth in presumed heterozygous females (Shokeir, 1971). During normal enamel formation the ameloblasts retreat from the dentine–enamel junction, each laying down a continuous column of matrix that soon starts to mineralize. On the basis of the single active X-chromosome hypothesis, it is assumed that the enamel stripes in X-linked hypoplasia heterozygotes represent regions formed by groups of ameloblasts in which the active X chromosome carried either the normal or the hypoplastic allele. An electron-microscope and microprobe study has in fact demonstrated two distinct types of sub-surface enamel corresponding to the externally visible stripes (Sauk, Lyon & Witkop, 1972). Hereditary abnormalities of the enamel occur in about 1/15000 individuals (Witkop, 1965).

The most common hereditary abnormality of the dentine is dentinogenesis imperfecta (hereditary opalescent dentine), an autosomal dominant condition occurring in 1/8000 of one population surveyed (Witkop, 1965). The dentine has an irregular structure, particularly away from the dentine–enamel junction, is poorly and irregularly mineralized and correspondingly softer than normal. The enamel often fractures under stress, perhaps because of poor support, exposing the soft dentine and leading to marked attrition of the tooth

crowns, sometimes down to gum level (Shokeir, 1972). In an extreme and striking variant of dentinogenesis imperfecta, known as shell teeth, the phase of dentinogenesis is greatly curtailed throughout the developing tooth, resulting in only a thin layer of dentine and a much larger pulp chamber than normal (Rushton, 1955). A condition similar to dentinogenesis imperfecta is frequently, though not always, seen in cases of osteogenesis imperfecta, a more generalized autosomal dominant disease where loose ligaments, deafness and blue sclerae may occur in association with fragility of the bones. It is thought likely that both dentinogenesis imperfecta and osteogenesis imperfecta are due to a primary abnormality in the organic component of the tissue involved, affecting the access of calcium and phosphorus to the sites of mineralization (Eastoe, Martens & Thomas, 1973). A rarer abnormality, restricted to the teeth, is dentinal dysplasia, also apparently due to an autosomal dominant gene, in which the roots of the teeth are much shorter than normal. The first formed dentine of the tooth crown is normal, but root dentine formation is apparently prevented by irregular and abnormal collagenous deposits (Rushton, 1955).

Inherited diseases with primary effects elsewhere in the body may also influence the formation of enamel and dentine, or cementum. One such condition is familial hypophosphataemia, an X-linked disease characterized by impaired renal reabsorption of inorganic phosphate, resulting in low serum phosphate concentrations and, in some cases, rickets or osteomalacia that do not respond to the normal therapeutic doses of vitamin D (Williams & Winters, 1972). The dentine is poorly calcified and may contain deficient tracts extending from the dentine–enamel junction to the pulp. The enamel above the tracts may be hypoplastic or cracked, providing access to the pulp chamber for oral micro-organisms. This can lead to multiple periapical abscesses associated with apparently normal and healthy-looking teeth (Archard & Witkop, 1966). In hypophosphatasia, probably an autosomal recessive condition, there is decreased serum alkaline phosphatase activity with inadequate mineralization of the bone matrix (Bartter, 1972). In addition, there may be almost a total lack of cementum and normally attached periodontal fibres, leading to poor support and premature loss of the teeth (Bruckner, Rickles & Porter, 1962).

5. Conclusions

Genetic variation can be found at each stage of dental development from the initiation of a tooth germ to the functional attachment of the resulting fully formed tooth in the bone of the jaw. Although many examples come from human studies, the conclusions are often of a rather general nature, more detailed, though from the human point of view indirect, information perhaps coming from work with experimental animals. Progress in human dental genetics has been frustrated in the past by a lack of adequate family data, though recent investigations and current work in the field indicate that this deficiency is being made good. It is to be hoped that more critical studies of human dental variation in the near future will provide better direct information about the control of tooth development in man.

References

Alvesalo, L. (1970) *The influence of sex-chromosome genes on tooth size in man* (Academic dissertation). Institute of Dentistry, University of Turku, Finland

Alvesalo, L. & Portin, P. (1969) *Acta Odontol. Scand.* **27**, 563–575

Archard, H. O. & Witkop, C. J., jr (1966) *Oral Surg. Oral Med. Oral Pathol.* **22**, 184–193

Bader, R. S. & Lehmann, W. H. (1965) *Am. Midl. Nat.* **74**, 28–38

Bartter, F. C. (1972) In: Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S., ed. *The metabolic basis of inherited disease*, 3rd ed., pp. 1295–1304. McGraw-Hill, New York & London

Bowden, D. E. J. & Goose, D. H. (1969) *J. Med. Genet.* **6**, 55–58

Brook, A. H. & Winter, G. B. (1970) *Br. Dent. J.* **129**, 123–130

Bruckner, R. J., Rickles, N. H. & Porter, D. R. (1962) *Oral Surg. Oral Med. Oral Pathol.* **15**, 1351–1369

Chung, C. S., Niswander, J. D., Runck, D. W., Bilben, S. E. & Kau, M. C. W. (1972) *Am. J. Phys. Anthropol.* **36**, 427–434

Danforth, C. H. (1958) *Genetics*, **43**, 139–148

Eastoe, J. E., Martens, P. & Thomas, N. R. (1973) *Calcif. Tissue Res.* **12**, 91–100

Garn, S. M., Dahlberg, A. A., Lewis, A. B. & Kerewsky, R. S. (1966) *J. Dent. Res.* **45**, 970

Garn, S. M., Lewis, A. B. & Kerewsky, R. S. (1965) *J. Dent. Res.* **44**, 439–441

Gorlin, R. J. & Pindborg, J. J. (1964) *Syndromes of the head and neck*. McGraw-Hill, New York & London

Grahnén, H. (1956) *Odontol. Revy*, suppl. no. 3

Grahnén, H., Lindahl, B. & Omnell, K. A. (1959) *Odontol. Revy*, **10**, 115–137

Grewal, M. S. (1962) *J. Embryol. Exp. Morphol.* **10**, 202–211

Grüneberg, H. (1963) *The pathology of development: a study of inherited skeletal disorders in animals*. Blackwell Scientific Publications, Oxford

Grüneberg, H. (1965) *J. Embryol. Exp. Morphol.* **14**, 137–159

Hitchin, A. D. & Morris, I. (1966) *J. Dent. Res.* **45**, 575–583

Jöhr, A. C. (1934) *Arch. Julius Klaus-Stift. Vererbungsforsch. Sozialanthropol. Rassenheig.* **9**, 73–131

Kraus, B. S. (1951) *Am. J. Hum. Genet.* **3**, 348–355

Lee, G. T. R. & Goose, D. H. (1972) *J. Med. Genet.* **9**, 336–339

Lundström, A. (1963) *Am. J. Hum. Genet.* **15**, 34–43

Mandeville, L. C. (1950) *Ann. Eugen.* **15**, 1–10

Niswander, J. D. & Chung, C. S. (1965) *Am. J. Hum. Genet.* **17**, 390–398

Pindborg, J. J. (1970) *Pathology of the dental hard tissues*. Munksgaard, Copenhagen

Pinto-Cisternas, J. & Figueroa, H. (1968) *Am. J. Phys. Anthropol.* **29**, 339–348

Rothhammer, F., Benado, M. & Pereira, G. (1971) *Hum. Biol.* **43**, 309–317

Rushton, M. A. (1955) *Ann. R. Coll. Surg. Engl.* **16**, 94–117

Sauk, J. J., jr, Lyon, H. W. & Witkop, C. J., jr (1972) *Am. J. Hum. Genet.* **24**, 267–276

Shokeir, M. H. K. (1971) *Clin. Genet.* **2**, 387–391

Shokeir, M. H. K. (1972) *Clin. Genet.* **3**, 442–447

Sofaer, J. A. (1969a) *Arch. Oral Biol.* **14**, 1213–1223

Sofaer, J. A. (1969b) *J. Embryol. Exp. Morphol.* **22**, 181–205

Sofaer, J. A. (1969c) *J. Embryol. Exp. Morphol.* **22**, 207–227

Sofaer, J. A. (1973a) *Dev. Biol.* **34**, 289–296

Sofaer, J. A. (1973b) *Evolution*, **27**, 427–434

Sofaer, J. A. (1974) *Genet. Res.* **23**, 219–225

Sofaer, J. A., Niswander, J. D., MacLean, C. J. & Workman, P. L. (1972) *Am. J. Phys. Anthropol.* **37**, 357–366

Sofaer, J. A. & Shaw, J. H. (1971) *J. Embryol. Exp. Morphol.* **26**, 99–109

Tsuji, T. (1958) *Jap. J. Hum. Genet.* **3**, 21–31

Williams, T. F. & Winters, R. W. (1972) In: Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S., ed. *The metabolic basis of inherited disease*, 3rd ed., pp. 1465–1485. McGraw-Hill, New York & London

Witkop, C. J., jr (1965) In: Tiecke, R. W., ed. *Oral pathology*, pp. 786–843. McGraw-Hill, New York & London

PRINTED IN GREAT BRITAIN BY WILLIAM CLOWES AND SONS LIMITED, LONDON, COLCHESTER AND BECCLES

APPENDIX 4C

Computation of Heritability Values

Since the genetic basis of a large proportion of cases of cleft lip with or without cleft palate (CL(P)) cannot be adequately explained, either in simple Mendelian terms or in terms of detectable chromosomal derangements, the problem becomes one of the relative importance of heredity and environment. The solution to such a problem can be arrived at only by using the methods of quantitative genetics and, in order to apply these methods, it is necessary to make certain assumptions about the underlying basis of the observed variation and to formulate some sort of model to explain it. In the present instance it seems that the appropriate model is that of quasi-continuous variation (2).

A quasi-continuous variable, in this case CL(P), is either present or absent but, when present, it varies continuously from the lowest level of expression to the highest. The accepted model of quasi-continuous variation is based on the assumption that there is an underlying scale of continuous variation of some quality (the result of a combination of all the genetic and environmental factors involved) which is immediately related to the development of the character. The character is absent in individuals who occupy a position on the scale below a critical threshold value and is present in those who occupy a position above it. The more the level on the underlying scale exceeds the threshold, the more intense is the expression of the character. A quasi-continuous character can therefore be regarded as a continuous variable whose expression has "visible" and "nonvisible" ranges. In the case of CL(P), the visible range starts just above the threshold, with slight notching of the lip, and extends through complete clefts of the lip and alveolus to complete clefts of the lip, alveolus and palate.

In a population of individuals, of whom some show the character and others do not, the distribution on the underlying scale is divided by the threshold. Assuming that the population is normally distributed, it is possible, by looking up standard tables of the normal distribution, to locate the mean of the population relative to the threshold in terms of the distribution's standard deviation. It is also possible to locate the mean of those individuals who fall above the threshold. The only information that is required is the proportion of the distribution that falls above (or below) the threshold (1).

In the general population CL(P) has an incidence of 0.1%. When we look up the appropriate tables (1), we find that the distance between the mean of the general population and the threshold, a distance which we can call x, is equal to 3.090 SD. The distance between the mean of the affected individuals and that of the general population, a distance which we can call a, is equal to 3.367 SD (see Figure 4C-1).

It should be clear that, if CL(P) has some hereditary basis, the frequency of this abnormality among relatives of CL(P) individuals should be higher than that among the general population. If, on the other hand, there were no hereditary basis and CL(P) were produced entirely by chance or by environmental factors, a group of relatives of CL(P) individuals would simply represent a random sample

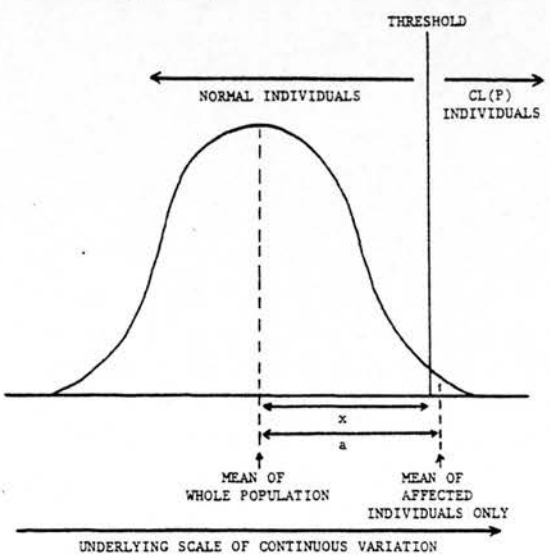


FIGURE 4C-1. For CL(P) in the general population, the proportion falling above the threshold is 0.1%; then x = 3.090 SD and a = 3.367 SD.

of the general population with a mean approximating that of the general population itself. Thus the difference between the mean of a group of relatives of CL(P) individuals and the mean of the general population, a difference which we can call d, is a measure of the degree to which CL(P) is a heritable character. This difference is simply the difference between the x value of the general population and a similar x value for the group of relatives (see Figure 4C-2).

The difference, d, has, however, to be considered in relation to the distance a and, also, to the degree of relationship (symbolized by r) of the relatives being studied. The distance a is the maximum value that d can assume, and this value would occur only if the relatives were monozygotic twins (genetically identical with the index cases, with $r = 1$) and if the character were entirely under genetic control. It should be pointed out here that, as the standard deviation is the only measure with which we can compare the positions of the general population and the group of relatives of affected individuals, and as we do not know what the variances of the two distributions are, we have to assume that both distributions (designated A and B) have the same standard deviation or variance (see Figure 4C-2).

The quantity which we are interested in calculating by all these manipulations is called the heritability and is symbolized by h^2. The heritability is the proportion of the observed variation which is due to additive genetic effects and is calculated from, and can be used to predict, the degree of resemblance between relatives. If the relatives we are dealing with are first degree relatives (full sibs, parents or children), then $r = 1/2$ as, on an average, these relatives have one-half of their genes in common with their index case. If they are second degree relatives (uncles, aunts, nephews and nieces) or third degree relatives (first cousins), then $r = 1/4$ and $1/8$, respectively, as second- and third degree relatives have, on an average, respectively, $1/4$ and $1/8$ of their genes in common with their index case. The maximum values of d when using first-, second- and third degree relatives are therefore $a/2$, $a/4$ and $a/8$, respectively, and these values would occur only if $h^2 = 1$. The actual value of the heritability is simply the difference d expressed as a proportion of its maximum possible value. Thus $h^2 = d/ar$.

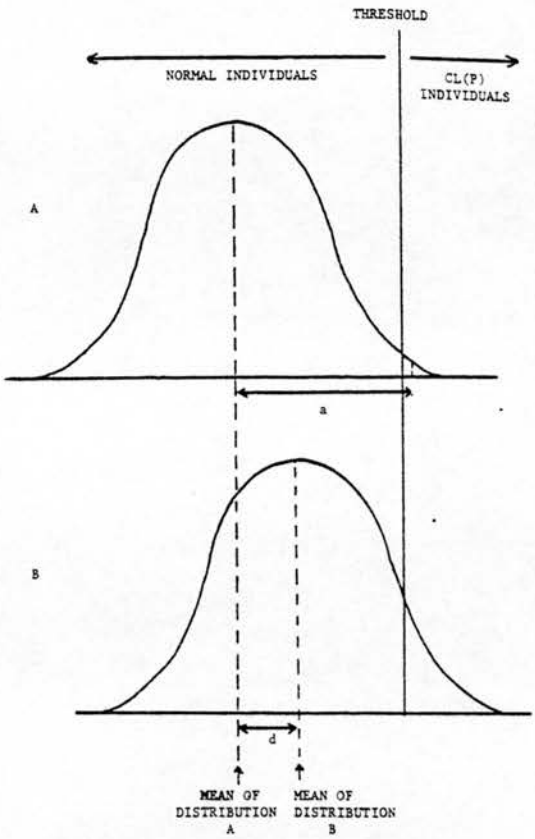


FIGURE 4C-2. Distribution A represents the general population. Distribution B represents relatives of affected individuals in distribution A. The assumption is that the two distributions have the same variance.

It should be mentioned that there are a number of complications and refinements associated with this method, and the intention here has been only to convey the principles involved. For further details, the reader is referred to Falconer (1).

J. A. SOFAER*

Bibliography

1. Falconer, D.S.: Ann. Hum. Genet., *29:* 51, 1965; 2. Grüneberg, H.: J. Genet., *51:* 95, 1952.

* Formerly Visiting Associate, Human Genetics Branch, National Institute for Dental Research, Bethesda, Maryland; Presently Nuffield Fellow, Department of Genetics, Cambridge University, Cambridge, England.

SECTION I

1. THE INFLUENCE OF HEREDITY

J. A. SOFAER

TERMS AND DEFINITIONS

Variation

When observing an organism, or when studying its development, it is important to realize that every character and every developmental process is subject to some degree of variation. No attribute, whether anatomical or physiological, normal or pathological, has an identical manifestation in all individuals, and there is no single rigid path of growth and development that is common to all members of a species. All characters therefore show some differences of nature or degree between individuals, and these differences are due partly to the inheritance of different genes and partly to chance and differences in the environment to which individuals have been subjected.

The first step in investigating the influence of heredity on growth and development is to observe the ways in which individuals differ with respect to any character of interest, and to study the patterns of variation of the character within families and within and between populations. A variety of analytical methods can then be applied to estimate the extent to which differences between individuals are due to genetic differences rather than to the influence of different non-genetic factors. Once something is known about the genetic contribution to the observed variation, established hereditary variants can be used to draw conclusions about developmental processes and their genetic control.

Differences occur not only between populations and between individuals but also, within individuals, between tissues or regions of the body. These differences begin to appear early in development when cells or groups of cells start to differentiate along a variety of developmental pathways. The basis for this differentiation seems to be that only particular genes have been "switched on" in particular cells, the majority of genes remaining inactive for most of the time. In certain circumstances it may be possible to compare gene activity in different tissues, and this can be a powerful way in which to study the effects of heredity on development.

Another kind of within-individual difference is that which occurs between paired structures on the two sides of the body. As hereditary potential and the general environment are expected to influence both sides of the same individual equally, asymmetry can be assumed to indicate lack of ability of a developing organ to buffer itself against chance developmental fluctuations and variation in its own local environment within the developing individual. The symmetry of bilateral structures is therefore an estimator of developmental stability, which, like any other character, is affected by both hereditary and environmental influences.

In addition to studying the variation of each character of interest separately, it may be useful to consider how two or more characters vary together. In the case of a syndrome of abnormalities produced by a single gene it is fair to assume that all characters showing some sign of abnormality are associated during development. When two characters that are correlated appear to be affected by several genes, it may be possible to estimate the degree to which their observed patterns of variation are due to common genetic influences and thereby draw conclusions about their developmental relationships.

Genes and Chromosomes

The variation observed among living things is composed of hereditary and environmental components. Heredity supplies the potential and the environment dictates the manner and degree to which this potential is expressed. The determinants of hereditary potential are the genes, each gene being an item of stored information that can be used to produce or control the production of a particular substance. The products of gene activity are ultimately partly responsible for all the physiological and anatomical properties of the individual. The fundamental property of the gene itself is its capacity for self-replication. The information contained in it can therefore be passed from one cell

generation to the next, and from a parent to its offspring. However, since genes cannot be observed directly, the existence of any gene can only be inferred from a study of variation in the character or characters it helps to produce.

The term **gene** is sometimes too general, and it may be more appropriate to talk of a **locus,** which is the site on a chromosome occupied by a gene, or an **allele,** which is one of a number of alternative forms that a gene may take. For example, at the MN blood group locus in man there may be either an "M-substance" producing allele or an "N-substance" producing allele. Allelic differences like this form the basis of the genetic component of variation, and, conversely, it is the possession of common alleles, derived from a common ancestor, that is responsible for the resemblance between relatives.

The nucleus of each human somatic cell normally contains 23 pairs of chromosomes. One member of each pair is derived from each parent so that each pair comprises a maternal and a paternal chromosome. One of the 23 pairs is a pair of **sex chromosomes** and the other 22 pairs are called **autosomes.** Each gamete contains only one member of each chromosome pair. At fertilization one gamete from each parent unite to form a single-celled **zygote** that subsequently develops into a new individual. As the zygote receives one chromosome of each pair from each parent, it is able to start its development with the full somatic complement.

The two members of each pair of autosomes are potentially identical in that the same loci are normally present in the same sequence in both. The only differences are allele differences that may be present at all, any or none of the loci. Each pair of autosomes is normally completely different from each of the others so that there are only two autosomal loci of each kind in each individual. Therefore, no matter how many alleles are available for a particular autosomal locus, no more than two will normally be present in each individual. If both alleles at a given locus are identical the individual is said to be **homozygous** at that locus for that allele, and if the alleles are different the individual is said to be **heterozygous.**

The sex chromosomes differ from the autosomes in that there are two alternative forms. One is the **X-chromosome,** which can be regarded as comparable to an autosome, and the other is the **Y-chromosome.** Loci carried by the X-chromosome are said to be **X-linked** or **sex-linked,** but no corresponding Y-linked loci have been demonstrated. The Y-chromosome can therefore be regarded as relatively inert. All viable individuals possess at least one X-chromosome, sex being dependent on whether the other member of the pair is another X-chromosome (which confers femaleness) or a Y-chromosome (which confers maleness). Females can therefore be either homozygous or heterozygous for all X-linked genes, but as males possess only one allele at each X-linked locus they can be neither. Males are consequently said to be **hemizygous** at all X-linked loci.

If an individual is homozygous (or hemizygous) at a given locus for any allele, then the observed effect is that of this allele alone. When an individual is heterozygous, and there are therefore two different alleles at the same locus, the outcome is dependent on the relationship between the alleles. If the two alleles are symbolized by A_1 and A_2, and in heterozygotes the observed effect is entirely that of A_2, then A_2 is completely **dominant** over A_1, and A_1 is completely **recessive** to A_2. All levels of dominance can occur from complete dominance of one allele over its partner to a situation of no dominance where each allele is expressed unaffected by the other.

Alleles are not absolutely stable entities. Although they are usually transmitted unaltered from generation to generation through the process of self-replication, rare events occur to cause changes within them. These events are called **mutations,** the new allele is a **mutant allele,** and individuals who show the effect of the mutant allele are known as **mutants.** The chromosomes themselves are also not entirely stable, and aberrations of the chromosomal complement, some of them associated with physical or mental abnormalities, may occur at low frequencies in all populations.

Genotype and Phenotype

The genetic constitution of an individual is known as his **genotype.** Genotype may refer to a specified locus or loci, or to all loci in general. An individual's **phenotype** is the final observed product of a combination of genetic and environmental influences. Phenotype may be used to refer to a specified character, or to the observable properties of the individual in general.

Different types of character can be thought of as being different distances from the fundamental level of gene activity. The further a character is removed from this fundamental genetic level, the greater the likelihood that its variation is dependent on allele differences at more than one locus, and also on environmental fluctuations. Enzymes, for example, are substances that are almost direct products of gene action, and in most cases it has been shown that the molecular structure of a single enzyme is dependent on a single gene. This means that variation in the structure, and consequently the function, of a particular enzyme is usually due to allele differences at a single locus. Morphological characters, on the other hand, are furthest from the fundamental genetic level and are the end results of a vast complexity of interacting developmental processes controlled by many genes and sensitive to external influences.

Since ontogeny has a basically diverging nature, the earlier an event occurs the more widespread its effects are likely to be. Each gene therefore probably affects many morphological characters, the breadth of its influence depending on the developmental stage at which it becomes active. It has in fact been observed that detectable single allele substitutions usually produce syndromes of morphological effects. Different aspects of such a syndrome may at first appear unrelated, but connections between them can often be established by probing back into their developmental relationships (Grüneberg, 1963).

TYPES OF VARIATION

Discrete Variation

Characters that show discrete variation exist in two or more qualitatively different forms. It is therefore not

possible to compare individuals by their measurements on a simple common scale. The best examples of discrete variables are found among biochemical or immunological characters such as blood groups. Here individuals are either of one blood type or another and there is no continuum of intermediates. Characters that exist in two forms only, such as sex, are examples of **dimorphisms;** and characters that exist in more than two forms, such as ABO blood type, are examples of **polymorphisms.** Variation within a population implies that not all individuals are of the same type. Variation between populations implies that the frequencies of the different forms in one population are different from those in another.

Discrete variation is often due to allele differences at a single locus. This can be demonstrated by studying the pattern of distribution of the character's different forms within families. The investigator compares the pattern with the theoretical expectations associated with different modes of genetic control, and by a process of elimination arrives at the most likely genetic hypothesis to explain the observations.

Continuous (Quantitative or Metric) Variation

Continuous variables, such as height, weight, tooth size and eruption time, are characters that can be measured against a continuous scale. In any range on any scale of measurement there is theoretically an infinite number of possible values, but for practical purposes it is usual to measure in terms of a chosen size of subdivision. For example, the mesiodistal diameter of mouse lower third molars can be measured to the nearest 1/100 mm. Variation within a population can then be expressed by the number of individuals that fall within each subdivision, and populations can be compared by the way in which individuals are distributed over all subdivisions of the scale (fig. 1).

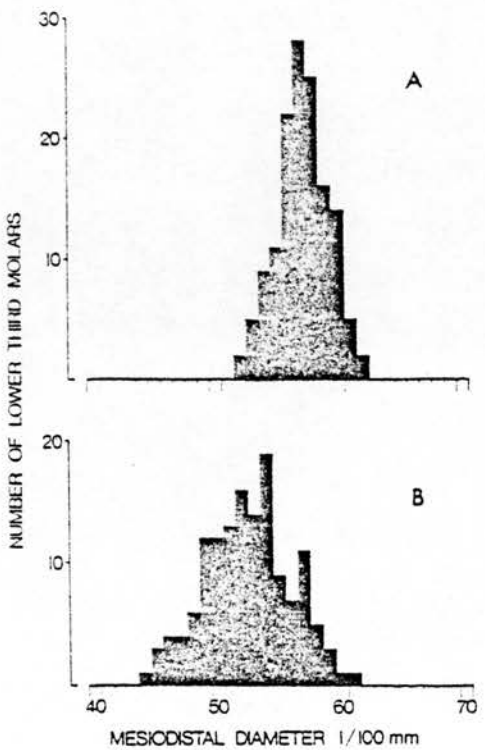


FIG. 1. Distributions of mesiodistal diameter of lower third molars in two genetically different groups of mice, A and B. The two groups differ with respect to both position and spread on the scale.

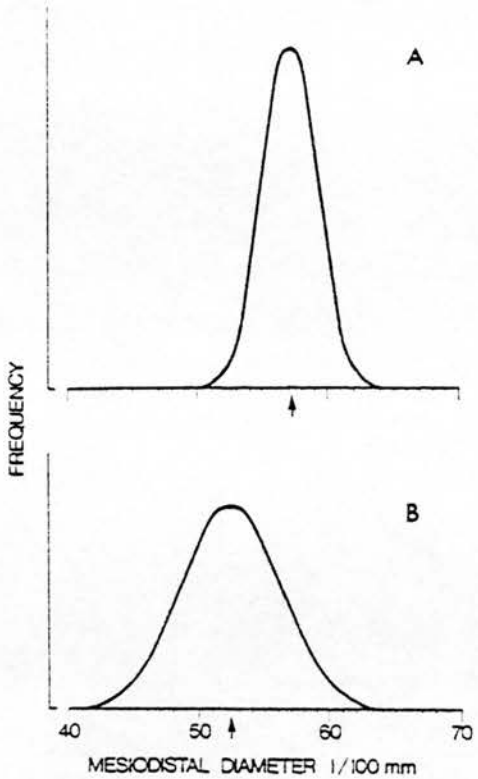


FIG. 2. Normal distributions corresponding to the data in Fig. 1. The means (indicated by arrows) of groups A and B are respectively 57.2 and 52.4, and the variances are 4.5 and 12.3.

The general form of each distribution in fig. 1 approximates to the most commonly encountered type of distribution, the **normal** distribution, the "ideal" shape of which is described by the normal curve. The position of such a distribution on the scale and the variation within it can be expressed in precise statistical terms as the mean and variance, and differences between populations can be established by a comparison of means and variances. The "ideal" distributions of the data in fig. 1 are shown as corresponding normal curves in fig. 2. The horizontal axis is still a scale of tooth diameter, but the vertical axis now measures the frequency with which teeth of a particular size occur in each group.

Continuous variables usually have a **multifactorial** basis: that is, several genes and environmental influences, each with a relatively small effect, contribute to an individual's

position on the continuous scale. Investigating the genetic control of continuous variables is then a problem of estimating the proportion of the observed variation due to genetic differences between individuals, and the proportion due to differences of environment. More specifically, it is a question of partitioning the observed or phenotypic variance, V_P, into its genetic and environmental components.

The total genetic variance is itself made up of a number of components, the most important being the **additive genetic variance**, V_A, which is the main cause of resemblance between relatives. The proportion of the phenotypic variance taken up by the additive genetic component is known as the **heritability**, and is symbolized by h^2. Thus: $h^2 = V_A/V_P$. Heritability is an expression of the reliance that can be placed on an individual's phenotype as an indication of the phenotype of his relatives. It is therefore useful when attempting to predict a course of development in a growing child, or the likelihood of his developing a disease for which there is a heritable predisposition.

The estimation of heritability depends on an analysis of resemblance between relatives. Consider a character that has no genetic component of variation. Phenotypic differences between individuals then have nothing to do with genetic differences and are due entirely to chance or environmental factors. Provided all individuals are exposed to the same environment, a group of related individuals is just as random a sample of the population as a group of unrelated individuals, as far as the character is concerned. Suppose, now, that the character does have some additive genetic component of variation. Related individuals, with more alleles in common than unrelated individuals, are expected to be more alike. The phenotypic variance of a group of related individuals is then lower than that of a random sample of the population, and these variances can be used to estimate the heritability of the character.

A common way of expressing the degree of resemblance between relatives is by a regression of offspring mean on **midparent value** (the mean of measurements of the character in the two parents of each family). If the two parental phenotypes are p_1 and p_2, the midparent value, $\bar{P}$, is then: $\bar{P} = (p_1 + p_2)/2$. Consider that $h^2 = 1$. All phenotypic variation is then due to additive genetic effects, and the phenotype of an individual is an exact indication of his genotype. Since each individual receives, on average, half his genetic information from one parent and half from the other, the mean of offspring, $\bar{O}$, is on average equal to: $\bar{O} = (p_1/2) + (p_2/2)$. Therefore $\bar{O}$ tends to equal $\bar{P}$, and if $\bar{O}$ is plotted against $\bar{P}$ for several different families the results average out as a straight line with a slope of 1. Thus, when $h^2 = 1$, the regression of offspring on midparent value, $b_{\bar{O}\bar{P}} = 1$; so that $h^2 = b_{\bar{O}\bar{P}}$. When $h^2 = 0$, and assuming that there is no environmental reason why offspring should be like their parents, each offspring is equivalent to a randomly selected individual from the general population, and the offspring means of all families vary around the mean of the general population. The results of plotting $\bar{O}$ against $\bar{P}$ then average-out as a straight line with zero slope. Thus, when $h^2 = 0$, $b_{\bar{O}\bar{P}} = 0$; so that in this case also $h^2 = b_{\bar{O}\bar{P}}$ (fig. 3). The equality of h^2 and $b_{\bar{O}\bar{P}}$ can in fact be shown to apply for all values of h^2.

Further details of these and other methods of estimating the heritability of continuous variables are given in Falconer (1964).

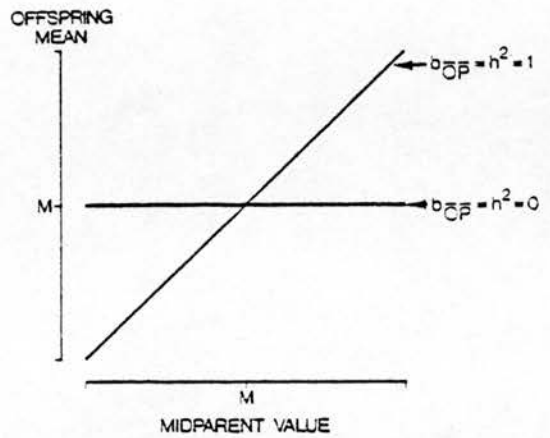


FIG. 3. The regression of offspring mean on midparent value. The two lines indicate the average relationships between parents and offspring for the two extremes of heritability. M is the mean value of the character in the population.

Quasi-continuous Variation

Characters that are either present or absent, but when present vary continuously, are known as quasi-continuous variables. The accepted model of quasi-continuous variation is based on the assumption that there is an underlying scale of continuous variation of some attribute (the result of a combination of all the genetic and environmental factors involved) that is immediately related to the development of the character. The character is absent in individuals who occupy a position on the scale below a critical **threshold** value, and present in those who occupy a position above it. The more the level on the underlying scale exceeds the threshold the more intense is the expression of the character. A quasi-continuous character can therefore be regarded as a continuous variable whose expression has a "visible" and a "non-visible" range.

In a population of individuals, some of whom show a quasi-continuous character and others of whom do not, the distribution on the underlying continuous scale is divided by the threshold. The shape of the whole distribution is therefore not disclosed, and unless there is reason to think otherwise it may be useful to assume it is normal. It is then possible, by consulting standard statistical tables of the normal distribution, to establish x, the distance of the threshold from the mean of the distribution in terms of σ, the distribution's standard deviation. The only information required is the proportion of the population, q, that falls above the threshold (fig. 4).

A simple comparison between populations can be made of means arrived at in this way, but such a comparison suffers from the possibly over-simplified assumption that the variances of all populations are the same. In order to compare variances as well as to make a more accurate comparison of means, the variable concerned must be capable

of being scored in three categories rather than two: non-affected, minimally affected, and moderately to maximally affected. In such a situation there are therefore two thresholds, and variances and means can be expressed in terms of what is assumed to be a constant interval between the two thresholds.

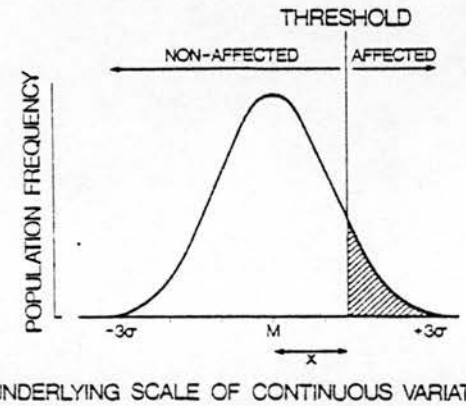


FIG. 4. The model of quasi-continuous variation. Only those individuals who fall above the threshold can be measured but the distribution on the underlying continuous scale is assumed to be normal. The distance, x, of the threshold from the mean, M, in terms of the standard deviation, σ, can be derived from tables given the proportion of affected individuals, q (hatched area as a proportion of the total area under the curve).

Figure 5 illustrates how such comparisons can be made. Applying proportions of each population to tables, as already described, q_{A1} will give x_{A1}, the distance of threshold 1 from the mean of distribution A; and q_{A2} will give x_{A2}, the distance of threshold 2 from the mean of distribution A. Both these distances are in terms of σ_A, the standard deviation of distribution A. A similar procedure can be adopted for distribution B. Thus the interval between the two thresholds, t, is equal to:

$$t = (x_{A2} - x_{A1})\sigma_A = (x_{B2} - x_{B1})\sigma_B$$

If for the sake of simplicity the distance between the two thresholds, t, is defined as unity, or one *threshold unit*, then the standard deviations of the two populations are equal to:

$$\sigma_A = 1/(x_{A2} - x_{A1}) \text{ threshold units,}$$
$$\sigma_B = 1/(x_{B2} - x_{B1}) \text{ threshold units,}$$

and the variances follow as σ_A^2 and σ_B^2. It follows also that the mean of distribution A:

$$M_A = -x_{A1}\sigma_A \text{ threshold units from threshold 1,}$$

and the mean of distribution B:

$$M_B = -x_{B1}\sigma_B \text{ threshold units from threshold 1.}$$

Greater detail of this kind of analysis is given in Falconer (1964, 1965).

Certain features of quasi-continuous variation are illustrated by a dental morphological variant in the mouse, a supernumerary cusp that has been found to occur at high frequency on the lower first molars of the Tuck No. 1

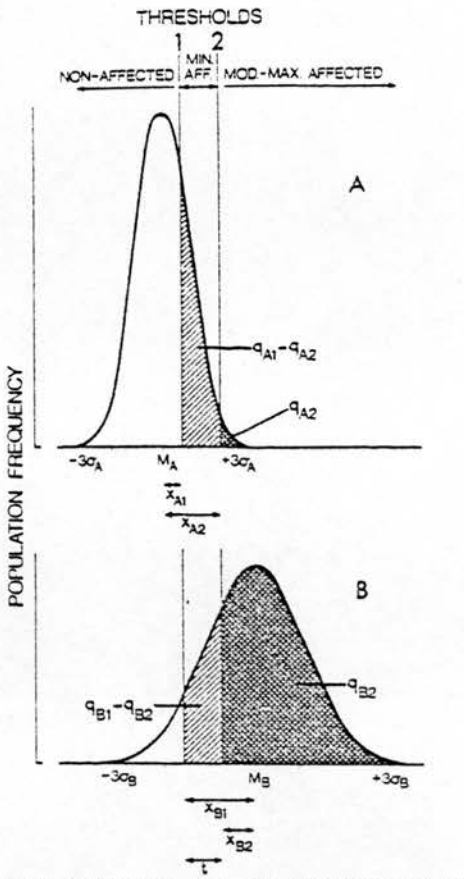


FIG. 5. Quasi-continuous variation with two thresholds. Values of x can be derived from tables given the appropriate proportion, q; and variances and means can be compared in terms of the threshold interval, t.

strain (fig. 6). Individual mice can be regarded as either non-affected (normal) or affected (variant), and affected animals can be classified according to four levels of expression of the cusp. Crosses of Tuck animals with other strains have resulted in groups of progeny with different frequencies of the cusp. Each group was genetically different from the others, and the different frequencies presumably reflect different mean levels of underlying genetic potential for cusp formation. These groups make it possible to test whether the relationship between the frequency of affected animals and the observed mean score of affected animals (observed MSA) conforms with what would be expected of normal distributions occupying different positions on the underlying continuous scale. The higher the frequency of affected individuals, the more severely they should be affected on average.

Just as it is possible to locate the mean of an entire distribution relative to the threshold, it is also possible to determine from tables the position of the mean of only those individuals who fall above the threshold. This, then, is the

theoretical position of the mean of affected individuals (expected MSA) based on the frequency of affected animals in each group. Expected MSA values were calculated for six groups of mice, making allowance for differences of variance by using the two-threshold model described above.

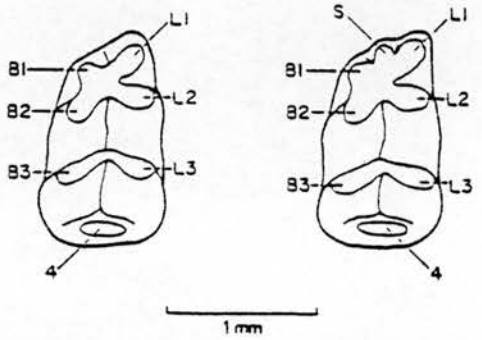


FIG. 6. Diagrams of occlusal surfaces of normal (left) and Tuck (right) lower left first molars. Both teeth have three buccal and three lingual cusps, B1–3 and L1–3, and a single distal cusp, 4. The Tuck tooth has an additional cusp, *S*. Reproduced with the permission of Pergamon Press Ltd. from Sofaer (1969a).

Figure 7 shows the relationship between observed and expected MSA values. There is a high correlation ($r = 0{\cdot}98$), and the regression ($b = 0{\cdot}86$) is not significantly different from 1. The mean levels of expression of the cusp in groups

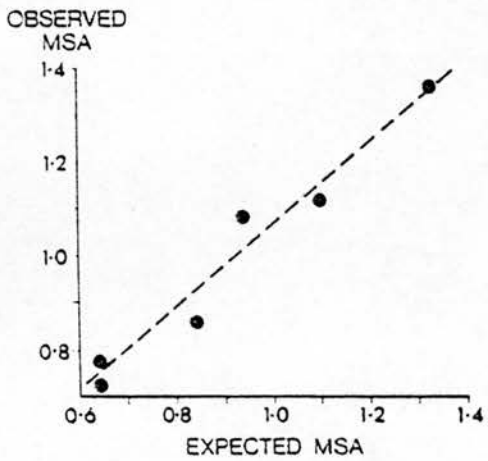


FIG. 7. The relationship between observed and expected MSA values for 6 groups of mice produced by crossing Tuck animals with different strains. Redrawn from Sofaer (1969a).

showing different frequencies of affected individuals therefore conform with what would be expected on the basis of the model of quasi-continuous variation.

As with continuous variables, the expression of a quasi-continuous character is usually dependent on a combination of many genes and environmental factors. It is therefore appropriate to be able to estimate the heritability, and, as with continuous variables, this is done by studying the resemblance between relatives. Consider the case of cleft lip with or without a cleft of the palate, CL(P). The assumption is that for each individual a combination of genetic and environmental influences determines the level of disposition to develop the malformation. An individual whose level falls below the threshold is normal, whereas one whose level falls above it is affected.

If CL(P) has no hereditary basis and is produced entirely by chance or environmental factors, then, provided all individuals are exposed to the same environment, a group of relatives of CL(P) individuals is equivalent to a random sample of the population with an incidence of the malformation approximating to that of the general population itself. On the other hand, if CL(P) is under some degree of genetic control the frequency of this abnormality among relatives of CL(P) cases should be higher than among the general population. Thus, assuming that there is no environmental reason why relatives should tend to be alike, *d*, the distance between the mean of a group of relatives of CL(P) individuals and the mean of the general population, is a measure of the degree to which CL(P) is a heritable condition. This is simply the difference between x_g and x_r derived from applying the proportions q_g and q_r to tables as described above (fig. 8). It should be pointed out here

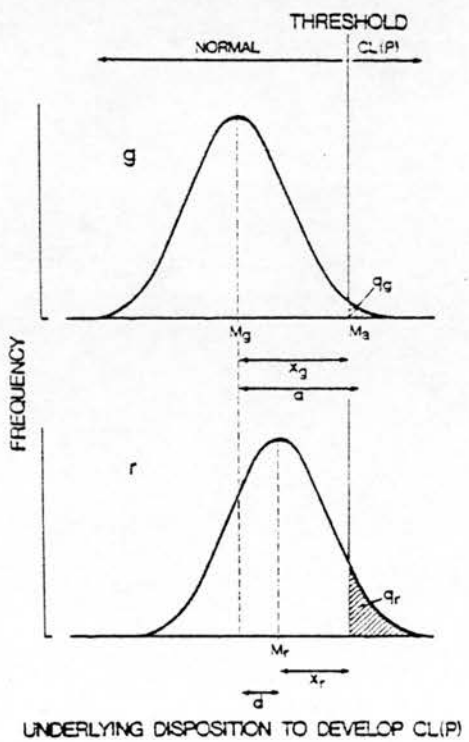


FIG. 8. Heritability estimation for a quasi-continuous character, CL(P). Distributions of the general population, g, and of a group of relatives of affected individuals, r, are shown. The values of x_g and a, and x_r, are derived from tables given the proportions q_g and q_r. The distance d, relative to a and the degree of relationship of the relatives used, provides the estimate of heritability.

that, as this is a single-threshold situation, it must be assumed that the variances of the general population and the group of relatives are the same.

The difference d has however to be considered in relation to a, the distance of the mean of affected individuals in the general population from the general population mean; and also to r, the degree of relationship of the relatives being used. The distance a is the maximum value that d can assume, and this can only occur if $h^2 = 1$ and if the relatives are monozygotic twins (genetically identical with their originally identified CL(P) cases, with $r = 1$). If the relatives used are first-degree (full sibs, parents or children), second-degree (aunts, uncles, nieces or nephews) or third-degree relatives (first cousins) then $r = \frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ respectively, as these relatives have on average $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ of their genes in common with their originally identified CL(P) cases. The maximum values of d when using first-, second- and third-degree relatives are accordingly $a/2$, $a/4$ and $a/8$, and these can only occur if $h^2 = 1$. The actual value of the heritability is simply the difference d expressed as a proportion of its maximum possible value. Thus: $h^2 = d/ar$ (Falconer, 1965).

Estimates of heritability for familial CL(P) from first-, second- and third-degree relatives by this method are respectively 0·83, 0·78 and 0·81 (Ross and Johnston, 1972). These values suggest that the differences between familial CL(P) cases and normal individuals could be largely due to the inheritance of different genes.

GENETIC VARIATION AND DEVELOPMENT

Normal Variation

The differences that are observed between "normal" individuals are often referred to as constituting **normal variation.** These differences may be due to allelic differences at single loci, as in the case of blood-group variants, and are then likely to be discrete differences; or they may have a multifactorial basis, as appears to apply to most dental characteristics, in which case variation is usually continuous or quasi-continuous. Investigating the genetic basis of normal variation in dental characteristics therefore depends largely on estimating the relative contributions of inherited and environmental differences to the observed differences between individuals. This is done by studying the degree of resemblance between relatives, which is often expressed as a heritability estimate for the character concerned. Since heritability is the ratio of the additive genetic variance to the total phenotypic variance, alteration of either the environmental or the genetic component will affect its value. This applies equally to any other expression of resemblance between relatives. Comparison of the resemblance between relatives for similar structures in the same population, such as neighbouring teeth, may therefore provide some information about the relative stability of the local environment around each developing structure within the developing individual as a whole.

Estimates of resemblance between relatives with respect to tooth size in man show that within each morphological class (incisors, premolars and molars) relatives tend to be most alike with respect to the teeth that develop early, and least alike with respect to those that develop late. That is, environmental variation contributes proportionally more to the observed differences between individuals in the later-developing teeth of each class (fig. 9, Table 1). Since, as is widely recognized, later-developing teeth show greater phenotypic variability, it follows that environmental variation also contributes more in absolute terms to the observed size differences between individuals in the later-developing teeth of each class. The point of interest here is what this pattern of hereditary *versus* environmental influence suggests about the way in which teeth develop. A possible interpretation is as follows. The early tooth of each class is the first to develop in its own region of the jaw and is therefore not initially in competition with any closely adjacent tooth germs. The later-developing tooth, on the other hand, must compete with already established tooth germs from the start, making do with what remains of any nutritional requirements that are necessary for growth. Consequently, variation in the supply of these requirements, within certain limits, is likely to affect the later rather than the earlier developing tooth of each class.

Abnormal Variation

The term **abnormal variation** is usually used to refer to gross differences from the population norm, many of which are due to single genes with major phenotypic effects, or to aberrations of whole chromosomes or parts of chromosomes. A study of the effect of a single gene on development generally consists of working back from the adult phenotype through to earlier and earlier stages, comparing normal and mutant individuals until there is no discernible difference between them. In doing this, it may be possible to formulate a hypothesis about the developmental basis of the mutant phenotype. It may even be possible to associate the observed abnormalities with a fundamental biochemical difference between normal and mutant individuals. On the other hand, a chromosomal aberration involves many loci, so there is unlikely to be a simple genetic cause for an associated syndrome of abnormalities. Investigation of a chromosomal aberration at the biochemical and even developmental level is therefore a more complex problem than the study of single genes.

Examples of single genes with major phenotypic effects in the mouse are the X-linked gene *tabby*, and two autosomal recessive genes, *crinkled* and *downless*. Each produces the same syndrome of abnormalities of hair, teeth and certain exocrine glands; all structures formed by the downgrowth of an epithelium into the underlying mesenchyme. These genes therefore presumably affect in some fundamental way the interaction that is known to occur between epithelium and mesenchyme during the formation of such structures, and, since the phenotypes they produce appear to be indistinguishable, their activities must be closely related during development.

Dental manifestations of the mutants include reduced size of the teeth and a characteristic mutant molar morphology (fig. 10), the rare occurrence of a supernumerary tooth just anterior to the first molar, and, very rarely, a composite tooth, apparently composed of incompletely

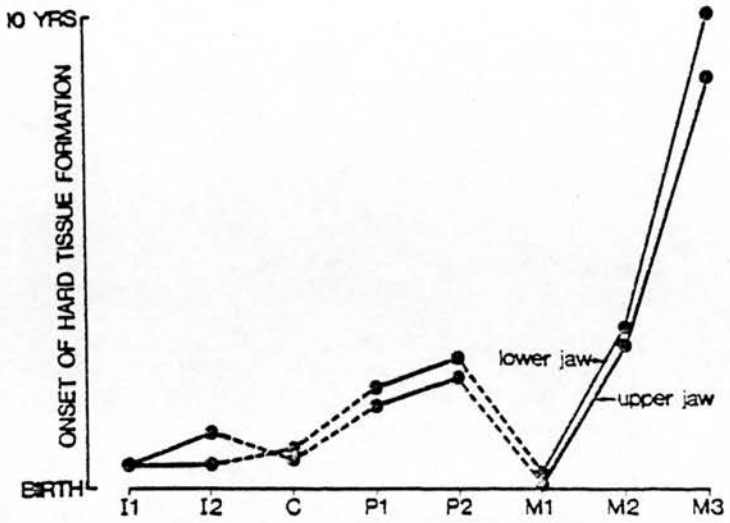


FIG. 9. The pattern of developmental timing (data from Orban, 1957) for incisors (I1 and I2), canine (C), premolars (P1 and P2) and molars (M1, M2 and M3). Solid lines connect points for teeth of the same class, and broken lines connect points for adjacent teeth of different classes.

TABLE 1

THE RESEMBLANCE BETWEEN RELATIVES WITH RESPECT TO MESIODISTAL TOOTH DIAMETER. THE DATA OF LUNDSTRÖM ARE RATIOS OF "HEREDITY/NON-HEREDITY" DERIVED FROM A COMPARISON OF IDENTICAL AND FRATERNAL TWINS; THOSE OF BOWDEN AND GOOSE ARE THE COMBINED CORRELATIONS FOR ALL PAIRS OF FIRST-DEGREE RELATIVES; AND THOSE OF SOFAER, BAILIT AND MACLEAN ARE CORRELATIONS BETWEEN FIRST-DEGREE RELATIVES

		Upper Jaw						
		I1	*I2*	*P1*	*P2*	*M1*	*M2*	*M3*
Lundström (1948)	Identical-twin pairs	91	90	62	53	—	—	—
	Fraternal-twin pairs	94	98	73	60	—	—	—
	Heredity / Non-heredity	3·9	2·8	5·1	2·8	—	—	—
Combined from Bowden and Goose (1969)	Pairs of first-degree relatives	308	284	—	—	—	—	—
	Correl. coeff.	0·49	0·38	—	—	—	—	—
Sofaer, Bailit and MacLean (1971)	Pairs of first-degree relatives	224	229	216	194	243	152	63
	Correl. coeff.	0·47	0·42	0·51	0·44	0·51	0·30	0·33
		Lower Jaw						
		I1	*I2*	*P1*	*P2*	*M1*	*M2*	*M3*
Lundström (1948)	Identical-twin pairs	93	95	80	62	—	—	—
	Fraternal-twin pairs	91	94	85	67	—	—	—
	Heredity / Non-heredity	3·3	3·5	3·6	2·5	—	—	—
Sofaer, Bailit and MacLean (1971)	Pairs of first-degree relatives	214	222	209	193	233	167	62
	Correl. coeff.	0·22	0·30	0·53	0·25	0·26	0·31	0·18

separate first molar and supernumerary elements. Embryological study has shown that the most fundamental mutant characteristic observed is a partial suppression of growth and differentiation of dental epithelium during a particular phase of development. The reduced size of the teeth and

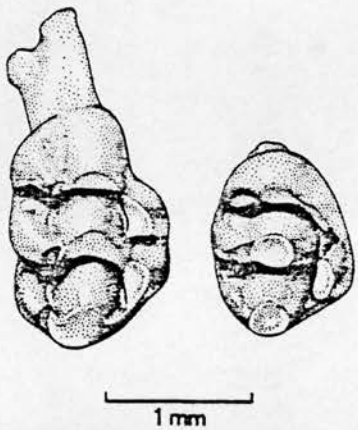


FIG. 10. Occlusal views of normal (left) and tabby (right) upper right first molars. Reproduced with the permission of Professor H. Grüneberg and The Company of Biologists Ltd. from Grüneberg (1965).

the mutant molar morphology seem to be due directly to the suppression of growth without an associated comparable delay in the onset of calcification, since the morphology of fully formed mutant molars corresponds in most respects to the morphology of an earlier stage of development in normal animals. The supernumerary tooth was found to arise independently from a normal extension of dental lamina anterior to the point of origin of the first molar, apparently as a response to small size of the first molar at the end of the suppression phase (fig. 11a and b). However, an overgrowth of dental lamina elicited at this stage does not necessarily progress to form a supernumerary tooth. Regression of the laminal downgrowth may occur, possibly as a result of competition with a first molar germ that has recently been released from the effects of the suppressive influence (fig. 11c). Since the supernumerary tooth arises independently, the composite teeth observed in adult animals are likely to be the result of fusion of the supernumerary and first molar germs. Thus, a complex pattern of dental abnormalities appears to result from a basically simple genetically controlled defect of epithelial function. This defect could be either intrinsic to the epithelium itself or secondary to an abnormality in the related mesenchyme.

These mutant mice do not provide the only example of an inherited association between fused and supernumerary teeth. Such an association can occur in the incisor region of dogs, and has led to the proposal of a mechanism whereby fusion might take place. It has been suggested that rapid growth of adjacent tooth germs can result in stripping of the external enamel epithelium from the dental lamina separating adjacent teeth, allowing the internal enamel epithelium of neighbouring germs freedom to come into contact and fuse. A further example is provided by the rice rat, a rodent native to the southern United States. In this case a supernumerary tooth sometimes develops posterior to the three molars of the normal series, and fusion may involve the first two molars, or all three molars of the normal series. Breeding records provide some evidence that the condition is caused by a single recessive gene. A section of a developing composite tooth composed of first, second and third molar elements is shown in fig. 12. It illustrates the bizarre result of fusion, the anterior end of the composite tooth germ showing an advanced state of histodifferentiation but the posterior end barely having progressed beyond undifferentiated internal enamel epithelium.

The reason for the association between supernumerary and fused teeth is not entirely clear, but may simply be related to the amount of space available to the developing tooth germs. Crowding of adjacent germs at a critical stage of development may predispose to epithelial stripping and subsequent fusion, and crowding may result from the presence of an additional tooth germ. Conversely, fusion, since it usually results in a smaller volume of developing tooth material than normal, may allow the lamina to proliferate further and form a supernumerary tooth. On the other hand, it could be that the basis of the association lies

FIG. 11(a). Tabby heterozygote lower first molar at 17 days of gestation showing a large bud of dental lamina anteriorly.
(b) Tabby heterozygote lower first molar at 19 days of gestation with a small supernumerary germ anteriorly.
(c) Lower first molar from the opposite side of the same animal as in (b) showing a laminal downgrowth that has failed to form a supernumerary tooth germ. (Anterior to the left.) Reproduced with the permission of the Company of Biologists Ltd. from Sofaer (1969b).

in the lamina itself, and that there is some inherited quality of the dental lamina predisposing to epithelial stripping and laminal hyperactivity.

In man, a relatively commonly occurring inherited abnormality is the absence of one or both upper lateral incisors. There is some evidence that the abnormality is due to a single gene, but the situation is not sufficiently clear cut to regard this as proved. In any event, it is certain that the condition is largely genetic in origin, the most informative kind of case perhaps being one in which an upper lateral incisor is absent on one side and present and of normal size on the other. In such a case, the two central incisors developed under different conditions, one having adjacent tooth germs on both sides, and the other having an adjacent germ on one side only. Unilateral absence therefore provides a simple situation in which to study the effect of local competition between tooth germs during development. In a sample of over 13,000 school-children from Hawaii unilateral absence, with a normal lateral on the other side, was observed in 77 cases. Measurements of the widths of the central incisors showed that the centrals tended to be larger on the side where the lateral was missing than on the side where the lateral was present and of normal size. This hereditary variant has thus revealed an association between local competition during development and final tooth-size.

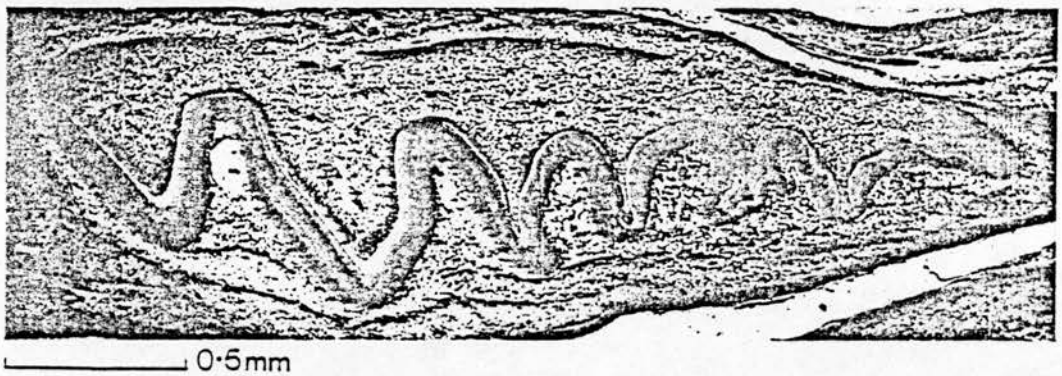


FIG. 12. Rice rat fused upper molar at one day after birth composed of first, second and third molar elements. (Anterior to the left.) Reproduced with the permission of The Company of Biologists Ltd. from Sofaer and Shaw (1971).

Within-Individual Differences

(a) Tissue and Regional Specificities

Differences may occur within individuals between tissues or regions of the body in terms of their developmental potencies. These potencies appear to be under the control of different sets of genes that have become activated in the different tissues. Variation in developmental potency is illustrated by studies of epidermis-dermis interaction that have involved separation of epidermis and dermis and the recombination of epidermis and dermis from different sources, allowing continued growth in culture. The results of such recombination procedures indicate that dental papillae from early tooth germs of mouse embryos are able to cooperate with lip furrow epithelium, and even with epidermis from the plantar surface of the foot, to form teeth; whereas the enamel organ becomes a stratified keratinizing epithelium when confronted with plantar surface dermis. The specificity for continued dental development therefore seems to reside in the dental papilla rather than the enamel organ at this stage (Kollar, 1972).

Direct evidence of differential gene action in the different tissues may be forthcoming if a suitable mutant affecting the system is available. Instead of making reciprocal recombinations between epidermis and dermis from different regions it is then possible to make combinations of normal epidermis with mutant dermis, and *vice versa*. In the case of *downless* mice, the dental abnormalities of which have already been mentioned, a simple difference between normal and mutant animals caused by the abnormality of epidermis-dermis interaction presents itself in the tail. The tails of normal mice are covered with hair whereas those of the mutants are bald (fig. 13). This results from suppression of formation of hair follicles in mutant tails, and could be

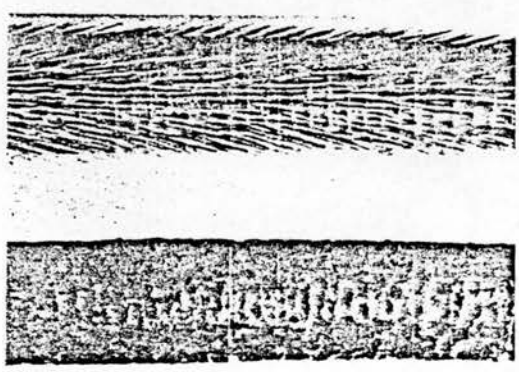


FIG. 13. Segments of normal (above) and downless homozygote (below) adult tails.

due to a failure in either the epidermal or dermal component of the system. Recombinations between epidermis and dermis from embryonic tails of *downless* homozygotes (phenotypically mutant) and *downless* heterozygotes (phenotypically normal) have been made at a stage well before the first signs of tail hair follicle formation in normal mice. After further development in culture, recombinations containing *downless* heterozygote epidermis and *downless* homozygote dermis produced hair follicles, whereas those containing *downless* homozygote epidermis and heterozygote dermis did not. Phenotypically normal epidermis was therefore required for follicle initiation, and mutant dermis did not prevent initiation from taking place. The mutant defect therefore appears to be restricted to the epidermis. Thus it seems reasonable to suppose that all the abnormalities shown by *downless* mice are due to a primary epithelial defect resulting from the presence of the mutant gene.

An example of regional rather than tissue specificity is the difference of morphology shown by different teeth along the length of the jaw. These differences have been explained in terms of **developmental fields** that may be related to gradients of evocating substances along the length of the developing tooth row. It is assumed that all prospective tooth germs have identical **prepatterns**, that is to say each is competent to form a complete set of morphological components in a prescribed relationship; but that the presence and relative size of each component depends on the level of the appropriate gradient at the position in which a tooth germ finds itself. Both prepatterns and gradients are presumably under genetic control, so that a study of variation in morphological fields may help to indicate how they are established during development.

(b) **Asymmetry**

The difference between sides within individuals with respect to a bilaterally represented character is of considerable interest in the study of development. As already noted, the level of asymmetry can be taken as a measure of developmental instability. Numerous studies using experimental animals have shown that normal phenotypes are relatively stable, or, put another way, normal development is narrowly canalized. On the other hand, if a major genetic or environmental influence acts to produce an abnormal phenotype, the abnormal phenotype is frequently much more variable. Abnormal development is therefore usually poorly canalized, and is accordingly associated with a relatively high level of asymmetry. In the case of an inherited malformation it is of interest to know whether the instability of development is restricted to the areas of the body primarily affected by the abnormality, or whether poor canalization is a general feature of development of affected individuals.

Two measures of developmental instability have been made in individuals suffering from familial CL(P) (cleft lip with or without cleft palate). As seen above, this condition has a high heritability, indicating that differences between CL(P) and normal individuals are largely due to the inheritance of different genes. The first measure of instability was the asymmetry of the lower first molar teeth. Although part of the same developmental complex as that affected by the malformation, these teeth are remote from the site of the cleft. The second measure was the asymmetry of the *atd* angle, a feature of the dermatoglyphic pattern of the palm of the hand. The *atd* angle is clearly far removed in developmental terms from the lip, alveolus and palate. Both the lower first molar and the *atd* angle showed greater asymmetry in familial CL(P) cases than in a control group, suggesting that developmental instability is not restricted to the area immediately related to the cleft. The interpretation of this finding was that there is a system of normal genes that buffers development against environmental fluctuations, that replacement of these normal genes by deleterious alleles lowers developmental stability, and that when buffering becomes too low to compensate for adverse environmental influences a major malformation in a particularly sensitive region may occur.

Animal experiments have shown, too, that developmental stability is reduced not only by major genetic or environmental influences but also through **inbreeding**. That is, as a result of a population becoming progressively more homozygous over all loci by the mating of closely related individuals, the phenotype becomes more unstable. Rapid progress towards a high level of homozygosity in laboratory animals is achieved by successive generations of brother-sister mating. In human populations lower levels of inbreeding may result from cousin marriages, or may occur in small isolated communities where potential mates are likely to be related. Instability may follow for two reasons. Firstly, homozygosity allows the expression of deleterious recessive alleles whose effects are masked by normal dominant counterparts in heterozygotes; and secondly, homozygosity provides the developing organism with a poorer choice of genes with which to deal with fluctuations of the developmental process. In keeping with the experimental findings is the association with inbreeding of an increased variance of tooth size and a suggestion of increased dental asymmetry in man.

By comparing different populations, it may be possible to learn more about the relative contributions of the genetic and environmental sources of instability. Four populations subject to different environmental conditions have been studied with this in mind. These were: natives of Tristan da Cunha, two distinct groups from the Solomon Islands (Kwaio and Nasioi), and Boston school-children. Dental asymmetry was found to be greatest in the population subject to the greatest environmental stress (Tristanites), as assessed by a consideration of climate, housing, diet and disease experience; and progressively lower in those subject to lower levels of environmental stress (Kwaio—Nasioi—Boston school-children). The measure of asymmetry used was the intraclass correlation between the mesiodistal diameters of antimeric teeth, and for all but one of the populations (Boston school-children) correlations for several pairs of teeth could be combined into a mean z score for each individual. The frequency distributions of individual z scores of males are shown for the other three populations in fig. 14. A lower z score means lower correlation between sides and therefore greater asymmetry. There is clearly a definite separation between the populations.

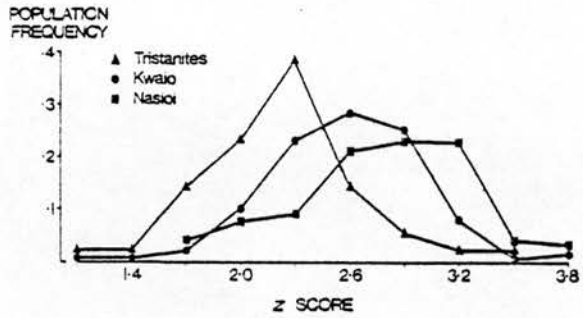


FIG. 14. Frequency distributions of individual z scores expressing dental asymmetry in three populations. Redrawn from Bailit, Workman, Niswander and MacLean (1970).

Correlated Characters

Correlation between characters in adult individuals may be the result either of common genetic control or of common environmental influence, or a combination of both. Associations between various dental characteristics have been discussed in the past; for example, third molar agenesis appears to be related to agenesis of other teeth and to retarded development. However, although plausible explanations may be advanced as to the basis of any association, it is not possible to determine the underlying nature of the observed correlation unless family data are available. Through an analysis of the resemblance between relatives, comparable to that by which heritabilities are estimated, a **genetic correlation** between characters can be calculated (Falconer, 1964). This is an expression of the degree to which characters are associated because they are influenced by the same genes. A high genetic correlation indicates a high degree of common genetic control, whereas a low genetic correlation implies that the characters vary together largely because of common environmental influences during development.

REFERENCES

Bailit, H. L., Workman, P. L., Niswander, J. D. and MacLean, C. J. (1970), "Dental Asymmetry as an Indicator of Genetic and Environmental Conditions in Human Populations." *Hum. Biol.*, **42**, 626.

Bowden, D. E. J. and Goose, D. H. (1969), "Inheritance of Tooth Size in Liverpool Families," *J. med. Genetics*, **6**, 55.

Falconer, D. S. (1964), *Introduction to Quantitative Genetics*. Edinburgh: Oliver and Boyd.

Falconer, D. S. (1965), "The Inheritance of Liability to Certain Diseases, Estimated from the Incidence Among Relatives," *Annals Hum. Genetics*, **29**, 51.

Grüneberg, H. (1963), *The Pathology of Development*. Oxford: Blackwell.

Grüneberg, H. (1965), "Genes and Genotypes Affecting the Teeth of the Mouse," *J. Embryology and Experimental Morphology*, **14**, 137.

Kollar, E. J. (1972), "Histogenetic Aspects of Dermal-Epidermal Interactions," in *Developmental Aspects of Oral Biology* (Slavkin and Bavetta, Eds.). New York and London: Academic Press.

Lundström, A. (1948), *Tooth Size and Occlusion in Twins*. Basle and New York: S. Karger.

Orban, B. J. (1957), *Oral Histology and Embryology*, 4th edition. St. Louis: The C. V. Mosby Company.

Ross, R. B. and Johnston, M. C. (1972), *Cleft Lip and Palate*, Chap. 3. Baltimore: The Williams and Wilkins Company.

Sofaer, J. A. (1969a), "The Genetics and Expression of a Dental Morphological Variant in the Mouse," *Arch. oral Biol.*, **14**, 1213.

Sofaer, J. A. (1969b), Aspects of the Tabby-Crinkled-Downless Syndrome. I. The Development of Tabby Teeth. II. Observations on the Reaction to Changes of Genetic Background. *J. Embryology and Experimental Morphology*, **22**, 181 and 207.

Sofaer, J. A., Bailit, H. L. and MacLean, C. J. (1971), "A Developmental Basis for Differential Tooth Reduction During Hominid Evolution," *Evolution*, **25**, 509.

Sofaer, J. A. and Shaw, J. H. (1971), The Genetics and Development of Fused and Supernumerary Molars in the Rice Rat. *J. Embryology and Experimental Morphology*, **26**, 99.

Chapter 2 – Single Gene Disorders

J. A. SOFAER

Some Fundamentals of Genetics · A Classification of Single Gene Disorders with Oral Manifestations · Single Gene Disorders Affecting the Maxilla and Mandible · Single Gene Disorders with Particular Effects on the Lips, Tongue or Gingiva · Single Gene Disorders Affecting the Oral Mucosa and Underlying Soft Tissues · Single Gene Disorders Affecting the Teeth · Single Gene Disorders with Functional or Neurological Manifestations · Index of Single Gene Disorders

Most genetic disorders fall into one of three broad categories. There are disorders associated with *chromosomal abnormalities*; those produced by *single abnormal genes*; and those with *multifactorial* aetiologies, due to the combined effects of several genes with a variable contribution from the environment. In addition, there are certain disorders in which the same clinical picture can be produced by different genetic mechanisms, and/or in which different forms are recognizable on clinical or biochemical grounds. In such situations, there is said to be *heterogeneity*.

This chapter is concerned only with disorders for which there is irrefutable evidence, or at least reasonably good evidence, for single gene control. More than 150 single gene disorders are included, classified in terms of their sites of action in the mouth. Despite this large number, most of the inherited immunological disorders and blood disorders affecting the mouth have been omitted. These are considered in Chapters 4 and 10.

SOME FUNDAMENTALS OF GENETICS

Genes and Chromosomes

Genes are determinants of hereditary characteristics. Each gene is an item of stored information that either specifies the structure of a particular substance or can be used to control the activity of other genes. Genes also have the capacity for accurate self-replication. The information contained in them can therefore be passed unaltered from one cell generation to the next, and from a parent to its offspring.

The term 'gene' is sometimes used rather loosely, and it is often better to be more specific. A *locus* is the site occupied by a gene. Loci are arranged linearly along each chromosome. An *allele* is one of a number of alternative forms that a gene may take, and different forms of the same gene are said to be *allelic*. For example, at the achondroplasia locus the normal allele is necessary for the production of normal cartilage, whereas the abnormal allele leads to the production of cartilage with abnormal growth properties.

In biochemical terms, an allele is a unique nucleotide sequence, and its locus is the position of this sequence in the very much longer linear series of nucleotides of a chromosome's DNA. The difference between one allele and another at the same locus may be limited to only one nucleotide position in the sequence, or it may occur at more than one position.

The nucleus of each human somatic cell normally contains 23 pairs of chromosomes. One member of each pair is contributed by each parent so that each pair can be said to comprise a maternally derived and a paternally derived chromosome. The two members of a pair are known as homologous chromosomes or *homologues*. One of the 23 pairs is a pair of *sex chromosomes*, and members of the other 22 are called *autosomes*. Each gamete contains only one member of each chromosome pair. At fertilization one gamete from each parent unite to form a single-celled *zygote* that subsequently develops into a new individual. As the zygote receives one member of each chromosome pair from each parent, it is able to start its development with the full somatic complement of chromosomes.

The two members of each pair of autosomes are potentially identical in that the same loci are present in the same sequence in both. The only differences are allele differences. Each pair of

autosomes is normally completely different from each of the others so that there are only two homologous autosomal loci of each type in a diploid cell, one on each member of a chromosome pair. If both alleles at a given locus are identical (it is usual to use 'locus' to refer to a pair of homologous loci) the individual is known as a *homozygote*, or is said to be *homozygous* at that locus for that allele; and if the alleles are different the individual is known as a *heterozygote*, or is said to be *heterozygous* at that locus.

The sex chromosomes differ from the autosomes in that there are two quite different forms. One is the *X-chromosome*, which can be regarded as comparable to an autosome. Loci carried by the X-chromosome are said to be *X-linked*, but are not necessarily directly concerned with sexual differentiation. The other is the much shorter *Y-chromosome*. All viable individuals possess at least one X-chromosome. The sex of an individual is dependent on whether the other member of the sex chromosome pair is another X-chromosome, associated with femaleness, or a Y-chromosome, which confers maleness. The Y-chromosome therefore carries genes essential for male sexual differentiation, but no Y-linked loci corresponding to those on the X-chromosome have been demonstrated. Females can thus be either homozygous or heterozygous for any X-linked gene, just as for an autosomal gene, but as males possess only one allele at each X-linked locus they can be neither. Males are consequently said to be *hemizygous* at all X-linked loci.

The Special Case of X-linked Genes

Over the past few years there has been increasing interest in why males, with only one copy of each X-linked gene, have similar characteristics to females, with a double dose of each X-linked gene. More specifically, heterozygotes for enzyme deficiencies controlled by autosomal genes (such as the deficiency of phenylalanine hydroxylase that causes phenylketonuria) generally have half the enzyme activity of normal homozygotes, whereas enzymes controlled by X-linked genes, such as G6PD, usually show the same level of activity in normal males and females, despite the difference in X-linked gene dosage. It is widely accepted that *dosage compensation* results from random inactivation of one or other of the X-chromosomes in each somatic cell of females at an early stage of embryonic development, so that only one of the two alleles at each locus is able to function in any cell. This action is irreversible, and the same chromosome remains inactive in all descendants of any given cell. Therefore, in a female heterozygous at an X-linked locus, about half the somatic cells have one allele active and the other half have the other allele active. This means that X-linked heterozygotes are *mosaics* made up of patches of two different cell types. This mosaicism may or may not be readily detectable.

The mosaicism situation can be illustrated by X-linked hypomaturation of the dental enamel, a form of amelogenesis imperfecta controlled by a single X-linked gene. Affected males, who have only the abnormal allele, possess only one type of somatic cell and produce uniformly abnormal enamel. Heterozygous females, with the normal allele on one X-chromosome and the abnormal allele on the other, have two types of somatic cell as a consequence of X-inactivation. In one, the X-chromosome carrying the normal allele is active, and these cells produce normal enamel. In the other, the X-chromosome carrying the abnormal allele is active, and these cells produce hypomature enamel. Heterozygous females therefore show patchiness of the enamel, with areas of normal translucent enamel side by side with areas of opaque, rather soft, hypomature enamel. The two types of area may occur as alternating irregular vertical bands. These bands probably reflect the pattern of proliferation and migration of cells of the internal enamel epithelium during growth of the tooth germ, before they differentiate into ameloblasts and lay down enamel.

It should be mentioned, however, that mosaicism of this type requires that the products of the normal and abnormal alleles remain within the cells in which they are produced. If there is diffusion of gene product between cells having different alleles active, the mosaicism present at the chromosome level will not be observed.

The Origin of Abnormal Alleles

Alleles are not absolutely stable. Although they are usually transmitted unaltered from one generation to the next, rare events occur that cause changes within them. These events are called *mutations*, and an allele that has undergone such a change is transmitted in its new *mutant* form.

The processes giving rise to new alleles are essentially random, so that many mutants result in reduced *fitness*, a reduced ability to contribute progeny to the next generation. In this way, harmful genes tend to be eliminated so that only the more favourable new variants remain. This is *natural selection*, and is responsible for sorting out the best genes for a particular environment.

Nevertheless, because mutation occurs at every generation, disadvantageous alleles are always being produced. A balance between the production of disadvantageous alleles through mutation and their elimination by selection results in a permanent presence of harmful alleles in the population, albeit at a low frequency. It is some of these alleles that are responsible for single gene disorders.

Genotype and Phenotype

The genetic constitution of an individual is known as his *genotype*. Genotype may refer to a specified locus or loci, or to all loci in general. An individual's *phenotype* is the final product of a combination of genetic and environmental influences. Phenotype may be used to refer to a specified character, or to all the observable properties of the individual taken together.

Different types of character can be thought of as being different distances from the fundamental level of gene activity. Enzymes, for instance, are almost direct products of gene action, and in most cases where genetic variation of enzyme structure has been demonstrated it has been shown that a single locus is responsible for the structure of a single enzyme. The structure and consequently the function of an enzyme is therefore usually simply and directly related to allele substitutions at a single locus.

Morphological characters, on the other hand, such as the almost infinite number of dimensions that can be used to describe the shape of the face and jaws, are furthest removed from the fundamental genetic level and are the end results of a vast complexity of interacting developmental processes. Each gene is therefore likely to influence many morphological characters, so that a detectable single allele substitution, although producing a unitary effect at the biochemical level, almost always results in a syndrome of morphological abnormalities. When a gene is known to affect a number of different characters in this way its action is said to be *pleiotropic*. By probing back into the early development of a syndrome, it may be possible to demonstrate how seemingly unassociated abnormalities in the adult have a common basis.

Some genes produce the same phenotype under all known conditions, but the effects of others may be modified, either by the environment or by other genes, or both. When the same mutant gene produces an abnormal phenotype in some individuals but not in others, it is said to have incomplete *penetrance*. If, among individuals who show the abnormal phenotype, the degree of abnormality varies, the phenotype is said to have variable *expressivity*. Incomplete penetrance tends to be associated with variable expressivity.

Dominance and Recessivity

If an individual is homozygous at a given locus for any allele, then the observed effect can only be the effect of this allele alone. When an individual is heterozygous, and there are therefore two different alleles at the locus, the phenotype is dependent on the relationship between the alleles. If the two alleles are symbolized by A_1 and A_2, and in heterozygotes the observed effect is entirely that of A_2, then A_2 is completely dominant over A_1, and A_1 is completely recessive to A_2. A dominant mutant allele therefore produces its disorder even if accompanied by a normal allele. In other words, heterozygotes for a dominant disorder are affected. By contrast, heterozygotes for a recessive disorder, with one normal and one recessive mutant allele, are phenotypically normal. An individual is affected by a recessive condition only if homozygous for the mutant allele.

All levels of dominance can occur from complete dominance of one allele over its partner to a situation of no dominance where each allele is expressed, unaffected by the other. In cases where there is no dominance, heterozygotes therefore show a level of abnormality intermediate between the mutant homozygote and normal phenotypes. Levels of heterozygote expressivity between no dominance and complete dominance constitute *incomplete dominance*.

Patterns of Inheritance for Single Gene Disorders

Alleles are transmitted from one generation to the next in a generally predictable way. The distribution of a disorder within families is related to the pattern of inheritance of the alleles involved, and to their dominance relationships with each other.

Autosomal dominant disorders

Individuals affected by an autosomal dominant disorder are almost always heterozygous, and the vast majority of matings in which such a disorder is involved are those between a heterozygote and a normal individual. This is simply because the mutant allele is generally very rare, which makes heterozygotes correspondingly rare and matings

between two heterozygotes, virtually the only source of homozygotes, very rare indeed. Nevertheless, cases of probable homozygosity, at least for the more common autosomal dominant disorders, are reported from time to time.

An autosomal dominant disorder is passed down from one generation to the next without a break, provided it does not affect fertility. Individuals of either sex pass the disorder on to both their sons and daughters. On average, half the children of matings between a heterozygote and a normal individual are affected and the other half normal. The affected children can likewise pass the condition on to half their offspring, but non-affected children cannot. An autosomal dominant pedigree illustrating these points is shown in Figure 2–1a.

The main exception to these general rules is that sometimes a dominant disorder is found in the child of two apparently normal parents. This could of course be due to the fact that the child is not the true biological offspring of these two parents, but there are two other possible explanations. The first is that one of the parents does in fact carry the mutant allele but does not express it. In other words, the gene is not fully penetrant. The second explanation is that a new mutation has occurred. These two alternatives can usually be distinguished, since in cases of incomplete penetrance there is often evidence of the mutant allele's presence in the family in previous generations.

Autosomal recessive disorders

In autosomal recessive inheritance the majority of affected individuals have unaffected parents. These unaffected parents are almost invariably heterozygotes, though it is possible, but extremely unlikely, that such a parent could be normal, passing on a mutant allele because of a new germ cell mutation. An autosomal recessive disorder is almost never passed down from one generation to the next, since this could happen only if an affected individual were to marry a heterozygote or, again, someone in whose germ cells a new mutation had occurred. As before, both of these possibilities are extremely unlikely.

When both parents are heterozygous, an average of one quarter of their offspring are affected, one half heterozygous and apparently normal, and the remaining quarter homozygous for the normal dominant allele. Heterozygous individuals who appear normal are known as *carriers* of the mutant allele. Both sexes are equally affected. An important finding in recessive disorders is that the parents of an affected individual are more often related than the parents of individuals selected at random from the population. That is, affected individuals tend to be the offspring of consanguineous marriages. The reason for this is that the chance of homozygosity occurring at any locus increases with the number of genes that parents have in common.

The pedigree shown in Figure 2–1b illustrates autosomal recessive inheritance. The two affected individuals are the offspring of a marriage between first cousins, both of whom have inherited the abnormal recessive allele from the same common ancestor.

X-linked disorders

In *X-linked recessive* disorders it is usually only males who are affected. This is because males carrying the abnormal allele always show its effects, as it is present in the hemizygous state, whereas females carrying the abnormal allele are nearly always heterozygous, again simply because the abnormal allele is generally rare. The abnormality is passed from a father, through his daughters, all of whom are unaffected carriers, to an average of half the sons of these carriers. Half of the daughters of a carrier, on average, are also carriers. A consequence of this is that frequently the maternal uncles of an affected individual are also affected. The condition is never passed directly from father to son. That is, there is never male-to-male transmission. A pedigree illustrating X-linked inheritance is shown in Figure 2–1c.

In *X-linked dominant* disorders heterozygous females as well as hemizygous males are affected. The result of this is that all the daughters but still none of the sons of an affected male are affected, producing a greater number of affected females than males in the population. For a small number of X-linked dominant disorders hemizygous males are so severely affected that they do not survive. The only affected individuals are therefore heterozygous females, and the disorder is inherited through them only. It should be noted, however, that abnormal sex ratios can also occur in autosomal disorders, through a difference of penetrance between males and females. If a disorder occurs more frequently in one sex than the other, and assuming there is no simple genetic reason for this, it is known as *sex influenced*. If it never occurs in one sex, even though individuals of that sex carry the gene, it is said to be *sex limited*.

The degree of dominance shown by X-linked disorders is usually less predictable than in autosomal conditions. In X-linked disorders where neither 'dominant' nor 'recessive' is speci-

fied, heterozygous females generally show an intermediate and variable level of expressivity.

Some Factors Affecting the Frequency of Single Gene Disorders

A dominant mutation has an immediately apparent effect on the individual in whom it first occurs. If the effect is not a serious one, the individual will survive and probably reproduce, with a 50 per cent chance of passing on the abnormal allele to each of his or her offspring. If the allele is passed on, further affected individuals arise, and the original mutation becomes responsible for more than one case of the disorder. On the other hand, in situations where a dominant gene causes early lethality or complete infertility, a single mutation is responsible for only one case of the disorder. The frequency with which a dominant disorder appears in a population therefore depends firstly on the rate at which new mutations occur, and secondly on the likelihood that an affected individual will pass the mutant gene on to the next generation.

An obvious consequence is that dominant genes causing early lethality or infertility do not produce disorders that occur within families. (The chances of two or more independent mutations of the same sort in the same family are too small to be considered.) Such disorders therefore occur sporadically, and there is no direct evidence for their genetic basis. By contrast, dominant disorders that do not reduce fitness show the typical familial pattern already described.

Unlike dominant mutations, recessive mutations do not affect the individuals in whom they first occur, since these individuals almost invariably become heterozygotes. (The chance of a new mutation occurring in an already existing heterozygote is very small indeed.) Because the effects of a recessive mutation are apparent only in mutant homozygotes, and because the production of homozygotes depends on matings between two heterozygotes, which occur only infrequently, large numbers of deleterious recessive alleles can accumulate in a population with only the occasional appearance of an affected individual on whom natural selection may act.

For X-linked disorders the situation is an intermediate one. A new mutation is expressed immediately in males, but not, or at least not usually, in females.

In small isolated populations, known as *isolates*, unusually high incidences of genetic disorders are often found. One reason for this is that if a mutation is introduced into such a population, the individual who carries it automatically constitutes a much larger proportion of this small population than would have been the case had the mutation occurred in the general population at large. In the case of dominant disorders not reducing fitness, a

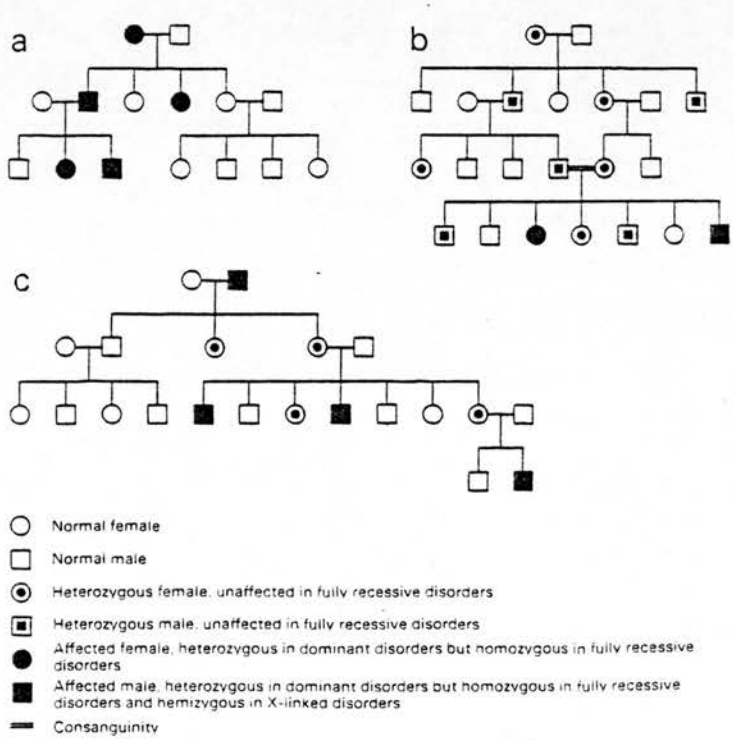


Figure 2–1. Pedigrees illustrating autosomal dominant (a), autosomal recessive (b) and X-linked (c) inheritance. Full descriptions are given in the text.

relatively high proportion of affected individuals is maintained in subsequent generations. In the case of recessive disorders, another factor comes into play. In small isolated populations a higher level of inbreeding than normal is inevitable, simply because of the limited choice of marriage partners. Since matings between related individuals increase the chance of homozygosity at any given locus among their offspring, and since there are likely to be some deleterious recessive alleles at relatively high frequencies in the population, unusually large numbers of individuals with recessive disorders may be produced.

A CLASSIFICATION OF SINGLE GENE DISORDERS WITH ORAL MANIFESTATIONS

The remainder of this chapter is devoted to a classification of single gene disorders that have oral manifestations. The classification is in the form of a descriptive catalogue divided into five parts (I, II, III, IV, V), each of which comprises a different number of sections. These are as follows.

I. Single gene disorders affecting the maxilla and mandible

I.A. Disorders in which there is almost always a cleft of the lip and/or palate
I.B. Disorders in which there is often a cleft of the lip and/or palate
I.C. Disorders producing hyperostosis, osteopetrosis, osteomas or odontomas
I.D. Disorders producing radiolucencies

II. Single gene disorders with particular effects on the lips, tongue or gingiva

II.A. Disorders affecting the lips
II.B. Disorders affecting the tongue
II.C. Disorders affecting the gingiva

III. Single gene disorders affecting the oral mucosa and underlying soft tissues

III.A. Disorders resulting in mucosal thickening or abnormal keratinization
III.B. Disorders resulting in nodular infiltration, fibrosis or deeper soft tissue lesions
III.C. Disorders resulting in papillomatosis
III.D. Disorders producing bullae and vesicles
III.E. Disorders producing vascular lesions
III.F. Disorders resulting in mucosal fragility
III.G. Metabolic disorders with associated oral ulceration
III.H. Disorders increasing susceptibility to candidosis

IV. Single gene disorders affecting the teeth

IV.A. Disorders affecting the number, size or shape of teeth
IV.B. Primary disorders of enamel formation
IV.C. Primary disorders of dentine formation
IV.D. Generalized disorders in which enamel and/or dentine formation may be abnormal
IV.E. Disorders in which cementum formation is abnormal
IV.F. Metabolic disorders resulting in tooth pigmentation
IV.G. Disorders with known manifestations in the dental pulp (excluding abnormal hard tissue formation)
IV.H. Disorders affecting the periodontium
IV.I. Disorders affecting tooth eruption
IV.J. Disorders in which there may be natal teeth

V. Single gene disorders with functional or neurological manifestations

V.A. Disorders affecting the temporomandibular joint and/or mandibular movement
V.B. Disorders with primarily neurological manifestations

Within each section, each disorder has been given a number according to where it falls alphabetically. The position of any disorder in the classification as a whole can therefore be specified by a catalogue number made up of a part number (Roman numeral), a section letter, and a number indicating the disorder's position within a section (Arabic numeral): for example, III.B.5. An index of disorders together with their catalogue numbers is given at the end of the chapter. Disorders producing a variety of effects on different oral structures appear in more than one section and therefore have more than one catalogue number.

Against each disorder listed in the body of the catalogue the mode of inheritance is indicated as autosomal dominant (AD), autosomal recessive

(AR) or X-linked (XL). An added asterisk, as for example in AR*, indicates that the mode of inheritance given can be regarded as fully proven. Absence of an asterisk indicates that the mode of inheritance given is only probable in the light of present knowledge of the disorder. Against disorders in which different clinical, biochemical or genetic forms can be recognized, the letter H, indicating heterogeneity, is given instead of a mode of inheritance. When this occurs, modes of inheritance of the different forms are given elsewhere.

For each disorder there is usually only a brief description, but selected references have been included so as to provide easy access to the most detailed information. There are four major reference works that include much more complete considerations of many of the disorders listed. These are: the fourth edition of *The Metabolic Basis of Inherited Disease*, edited by J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson, published by the McGraw-Hill Book Company of New York and London in 1978, referred to in this chapter as *MBID*; the fifth edition of *Mendelian Inheritance in Man*, by V.A. McKusick, published by the Johns Hopkins University Press of Baltimore and London in 1978, referred to in this chapter as *McK*; *Oral Facial Genetics*, edited by R.E. Stewart and G.H. Prescott, published by the C.V. Mosby Company of St Louis in 1976, referred to in this chapter as *OFG*; and the second edition of *Syndromes of the Head and Neck*, by R.J. Gorlin, J.J. Pindborg and M.M. Cohen, Jr, published by the McGraw-Hill Book Company of New York and London in 1976, referred to in this chapter as *SHN*. Where appropriate, references to these works are cited at the head of the bibliography list for each disorder. A number following *McK* is a McKusick's catalogue number, and numbers following *MBID*, *OFG* and *SHN* are inclusive page numbers. *OFG* and *SHN* also contain illustrations of many of the disorders. In addition, some of the disorders are described more fully elsewhere in this book.

In this chapter each description and bibliography list is given only once, even if the disorder appears in more than one section. Only the name of the disorder, its catalogue numbers, mode of inheritance, and reference to where in the classification its description and bibliography list can be found appear in additional sections of the catalogue.

Finally, before continuing with a consideration of the disorders themselves, it should be noted that any classification of genetic disorders is subject to change as more information becomes available. On one hand, the designation of a newly described disorder as a single genetic entity is not always final, since it may later become apparent that what was thought to be a single disorder is really a heterogeneous group of related conditions. On the other, newly described disorders that initially appear to be distinct may ultimately be included within a single syndrome as evidence accumulates to show that the syndrome in question has a wide range of expression.

SINGLE GENE DISORDERS AFFECTING THE MAXILLA AND MANDIBLE

I.A. Disorders in which There Is Almost Always a Cleft of the Lip and/or Palate

I.A.1. Brachial plexus neuritis and cleft palate
(AD*)

Cleft palate associated with deep set and hypoteloric eyes, and recurrent attacks of brachial plexus neuritis. Attacks of neuritis involve pain, weakness, wasting, depression of reflexes and sensory loss.

McK 16210 *OFG* 543 *SHN* 166

Erikson, A. (1974) Hereditary syndrome consisting of recurrent attacks resembling brachial plexus neuritis, special facial features and cleft palate. *Acta Paediatrica Scandinavica*, **63**, 885–888.

Jacob, J.C., Andermann, F. & Robb, J.P. (1961) Heredofamilial neuritis with brachial predilection. *Neurology*, **11**, 1025–1033.

I.A.2. Campomelic (camptomelic) dwarfism
(AR*)

A combination of flat face, ocular hypertelorism, micrognathia and cleft palate, associated with bowing of the long bones and other skeletal defects. The condition is frequently lethal in the neonatal period or during the first few months of life due to respiratory distress.

McK 21197 *OFG* 543 *SHN* 156

Schmickel, R.D., Heidelberger, K.P. & Poznanski, A.K. (1973) The camptomelique syndrome. *Journal of Pediatrics*, **82**, 299–302.

Spranger, J., Langer, L.O. & Maroteaux, P. (1970) The increasing frequency of a syndrome of multiple osseous defects? *Lancet*, **ii**, 716.

Storer, J. & Grossman, H. (1974) The campomelic syndrome. *Radiology*, **111**, 673–681.

I.A.3. Camptodactyly, cleft palate and club foot (AD)

Cleft palate in conjunction with club foot and a flexion contracture deformity of the fingers.

McK 11430 *OFG* 544 *SHN* 147

Gordon, H., Davies, D. & Berman, M. (1969) Camptodactyly, cleft palate and club foot. A syndrome showing the autosomal-dominant pattern of inheritance. *Journal of Medical Genetics*, **6**, 266–274.

I.A.4. Cerebrocostomandibular syndrome (AR*)

A combination of microcephaly, thoracic deformity (hypoplastic or absent ribs and vertebral anomalies) and the Robin anomalad (see I.A.17.).

McK 21400 *OFG* 543 *SHN* 149

Miller, K.E., Allan, R.P. & Davis, W.S. (1972) Rib gap defects with micrognathia. *American Journal of Roentgenology*, **114**, 253–256.

I.A.5. (II.A.3.) Cleft lip-palate, mucous cysts of the lower lip, popliteal pterygium, digital and genital anomalies (popliteal pterygium syndrome) (AD*)

Cleft lip-palate associated with genital and musculo-skeletal anomalies and a variety of abnormalities of the skin and oral mucous membranes. The most striking of these is a web of skin, extending from the heel to the ischial tuberosity, that limits movement of the leg. There may be pits, fistulae or mucous cysts of the lower lip, and abnormal cords or threads of mucous membrane connecting the upper and lower jaws, limiting intermaxillary opening. Similar epithelial strands may be found connecting the upper and lower eyelids. There may be hypoplasia or agenesis of the digits, and soft tissue syndactyly of the second to fifth toes.

McK 11950 *OFG* 539 *SHN* 121–124

Bixler, D., Poland, C. & Nance, W.E. (1973) Phenotypic variation in the popliteal pterygium syndrome. *Clinical Genetics*, **4**, 220–228.

Gorlin, R.J., Sedano, H.O. & Cervenka, J. (1968) Popliteal pterygium syndrome; a syndrome comprising cleft lip-palate, popliteal and intercrural pterygia, digital and genital anomalies. *Pediatrics*, **41**, 503–509.

Rintala, A. & Lahti, A. (1970) The facio-genito-popliteal syndrome. *Scandinavian Journal of Plastic and Reconstructive Surgery*, **4**, 67–71.

Rintala, A., Lahti, A. & Gylling, U. (1970) Congenital sinuses of the lower lip in connection with cleft lip and palate. *Cleft Palate Journal*, **7**, 336–346.

I.A.6. Cleft lip-palate, ocular hypertelorism and microtia (Bixler syndrome) (AR*)

Cleft lip-palate associated with ocular hypertelorism, hypoplasia of the external ear, mild microcephaly, ectopic kidneys and congenital heart defects.

McK 23980 *OFG* 538 *SHN* 142

Bixler, D., Christian, J.C. & Gorlin, R.J. (1969) Hypertelorism, microtia and facial clefting: a newly described inherited syndrome. *American Journal of Diseases of Children*, **118**, 495–500.

I.A.7. Cleft lip-palate with abnormal thumbs and microcephaly (AR)

Cleft lip-palate with microcephaly, hypoplastic and distally positioned thumbs, and shortened radii.

McK 21610 *OFG* 539 *SHN* 142

Juberg, R.C. & Hayward, J.R. (1969) A new familial syndrome of oral, cranial and digital anomalies. *Journal of Pediatrics*, **74**, 755–762.

I.A.8. Cleft palate and oral synechiae (AD*)

Cleft palate in combination with cord-like adhesions between the free borders of the palate and lateral parts of the tongue and floor of the mouth.

McK 11955 *OFG* 543 *SHN* 138–141

Fuhrmann, W., Koch, F. & Schweckendick, W. (1972) Autosomal dominante Vererbung von Gaumenspalte und Synechien zwischen Gaumen und Mundboden oder Zunge. *Humangenetik*, **14**, 196–203.

I.A.9. Cleft palate, deafness and oligodontia (AR)

Cleft palate with stapes fixation, reduction in number of deciduous teeth, absence of permanent teeth, and bony abnormalities of the foot.

McK 21630 *OFG* 544 *SHN* 147

Gorlin, R.J., Schlorf, R.A. & Paparella, M.M. (1971) Cleft palate, stapes fixation and oligodontia – a new recessively inherited syndrome. *Birth Defects*, **7**(7), 87–88.

I.A.10. Congenital spondyloepiphyseal dysplasia and cleft palate (AD*)

Congenitally reduced stature due primarily to shortness of the neck and trunk, but also to shortness of the extremities. There may be associated myopia and cleft palate.

McK 18390 *OFG* 546 *SHN* 141–142

Ginter, D.N. & Lee, S.O. (1974) Spondyloepiphyseal dysplasia congenita. *Birth Defects*, **10**(12), 379–382.

Spranger, J.W. & Langer, L.O. (1970) Spondyloepiphyseal dysplasia congenita. *Radiology*, **94**, 313–322.

I.A.11. (*III.H.2., IV.A.5., IV.D.5.*) *Ectro-dactyly-ectodermal dysplasia-clefting* (*EEC*) *syndrome* (AD*)

A combination of lobster claw deformity of the hands and feet (reduction in number of fingers or toes with syndactyly), nasolacrimal duct obstruction and cleft lip-palate. Scalp hair, eyelashes and eyebrows may be sparse, nails brittle and sebaceous glands absent. There may be anodontia or severe oligodontia with enamel hypoplasia. The oral mucosa is predisposed to candidosis.

McK 12990 *OFG* 564–566 *SHN* 118–120

Bixler, D., Spivack, J., Bennett, J. & Christian, J.C. (1971) The ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome. Report of two cases and review of the literature. *Clinical Genetics*, **3**, 43–51.

Bystrom, E.B., Sanger, R.G. & Stewart, R. (1975) The syndrome of ectrodactyly, ectodermal dysplasia and clefting (EEC). *Journal of Oral Surgery*, **33**, 192–198.

Pashayan, H.M., Pruzansky, S. & Solomon, L. (1974) The EEC syndrome. Report of six patients. *Birth Defects*, **10**(7), 105–127.

Penchaszadeh, V.B. & De Negrotti, T.C. (1976) Ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome. Dominant inheritance and variable expression. *Journal of Medical Genetics*, **13**, 281–284.

Rosenmann, A., Shapira, T. & Cohen, M.M. (1976) Ectrodactyly, ectodermal dysplasia and cleft palate (EEC syndrome). Report of a family and review of the literature. *Clinical Genetics*, **9**, 347–353.

I.A.12. Hypohidrosis, thin wiry hair, dystrophic nails and cleft lip-palate (AD)

Cleft lip-palate with hypohidrotic or anhidrotic ectodermal dysplasia and short stature.

McK 12940 *OFG* 540 *SHN* 144–146

Rapp, R.S. & Hodgkin, W.E. (1968) Anhidrotic ectodermal dysplasia: autosomal dominant inheritance with palate and lip anomalies. *Journal of Medical Genetics*, **5**, 269–272.

I.A.13. (*II.A.8., II.B.7., IV.A.14.*) *Oral–facial–digital syndrome I* (*OFD I syndrome*) (XL*)

Cleft palate associated with a median 'pseudocleft' of the upper lip, cleft or lobulated tongue, hypoplasia of the nasal alar cartilages, digital malformations and mental retardation. The pseudocleft of the upper lip is due to the presence of abnormal frenula that cause a severe reduction in the depth of the mucobuccal fold. Similar abnormal frenula may occur in other parts of the buccal sulcus of both upper and lower jaws. Ankyloglossia is sometimes present. There may be supernumerary teeth in the maxillary canine/premolar region, and absence of mandibular lateral incisors. The syndrome is inherited as an X-linked dominant trait, limited to females and apparently lethal in males.

McK 31120 *OFG* 557–560 *SHN* 562–567

Axrup, K., Lindquist, B. & Samuelson, G. (1971) Oral-facial-digital syndrome. *Odontologisk Revy*, **22**, 137–144.

Burzynski, N.J., Podruch, P.E., Dinno, N. & Snawder, K. (1975) Oral-facial-digital syndrome. *Oral Surgery*, **39**, 735–741.

Lauterstein, A. & Pruzansky, S. (1969) Tooth anomalies in the oral-facial-digital syndrome. *Teratology*, **2**, 137–146.

Melnick, M. & Shields, E.D. (1975) Orofaciodigital syndrome, type I. A phenotypic and genetic analysis. *Oral Surgery*, **40**, 599–610.

Whelan, D.T., Feldman, W. & Dost, I. (1975) The oro-facial-digital syndrome. *Clinical Genetics*, **8**, 205–212.

Yeamans, E.H. (1973) OFD I syndrome and mental retardation. *Cleft Palate Journal*, **10**, 84–91.

I.A.14. Oto-palato-digital (*OPD*) *syndrome* (XL*)

A combination of characteristic facies, conduction deafness, short stature and generalized bone dysplasia with cleft palate. The supraorbital ridges are prominent with the brow overhanging, antimongoloid obliquity of the palpebral fissures, ocular hypertelorism, a broad nasal root and depressed nasal bridge. The thumb and great toe are short and spatulate, and the fingers and toes irregular in form and direction of curvature.

McK 31130 *OFG* 561–564 *SHN* 592–595

Gall, J.C., Stern, A.M., Poznanski, A.K., Garn, S.M., Weinstein, E.D. & Hayward, J.R. (1972) Oto-palato-digital syndrome. Comparison of clinical and radiographic manifestations in males and females. *American Journal of Human Genetics*, **24**, 24–36.

Gorlin, R.J., Poznanski, A.K. & Hendon, I. (1973) The oto-palato-digital (OPD) syndrome in females. *Oral Surgery*, **35**, 218–224.

I.A.15. Pterygium syndrome (AR*)

Multiple pterygia (skin webs) involving the neck, fingers, and antecubital, popliteal and intercrural areas, associated with growth retardation and cleft palate.

McK 26500 *OFG* 545 *SHN* 527–529

Norum, R.A., James, V.L. & Mabry, C.C. (1969) Pterygium syndrome in three children in a recessive pedigree pattern. *Birth Defects*, **5**(2), 233–235.

I.A.16. Roberts syndrome (*cleft lip-palate and tetraphocomelia; Appelt syndrome*) (AR*)

Bilateral cleft lip-palate associated with tetraphocomelia, a reduction in digit number, ocular hypertelorism and growth deficiency.

McK 26830 *OFG* 538 *SHN* 125–127

Freeman, M.V.R., Williams, D.W., Schimke, N. & Temtamy, S.A. (1974) The Roberts syndrome. *Clinical Genetics*, **5**, 1–16.

I.A.17. Robin anomalad (cleft palate, micrognathia and glossoptosis; Pierre Robin syndrome) (H)

A subset of characteristics that may be present in a number of syndromes with a variety of aetiologies, some genetic. Among these are campomelic dwarfism (I.A.2.), the cerebrocostomandibular syndrome (I.A.4.), and the Stickler syndrome (I.B.8.). The main features are mandibular hypoplasia and cleft palate. The micrognathia results in poor support for the tongue, thereby allowing it to fall downwards and backwards (glossoptosis), causing partial respiratory obstruction and feeding difficulties. The Robin anomalad may also appear in conjunction with congenital heart malformations and club foot (X-linked), or as an isolated defect (autosomal recessive).

McK 26180 (AR), 31190 (XL) *OFG* 537, 549
SHN 132–136

Carroll, D.B., Peterson, R.A., Worton, E.W. & Birnbaum, L.M. (1971) Hereditary factors in the Pierre Robin syndrome. *British Journal of Plastic Surgery*, **24**, 43–47.
Gorlin, R.J., Cervenka, J., Anderson, R.C., Sauk, J.J. & Bevis, W.D. (1970) Robin's syndrome. A probably X-linked recessive subvariety exhibiting persistence of left superior vena cava and atrial septal defect. *American Journal of Diseases of Children*, **119**, 176–178.
Hanson, J.W. & Smith, D.W. (1975) U-shaped palatal defect in the Robin anomalad: developmental and clinical relevance. *Journal of Pediatrics*, **87**, 30–33.
Opitz, J. (1969) Familial anomalies in the Pierre Robin syndrome. *Birth Defects*, **5**(2), 119.
Randall, P., Krogman, W.M. & Jahina, S. (1965) Pierre Robin and the syndrome that bears his name. *Cleft Palate Journal*, **2**, 237–246.

I.A.18. X-linked cleft palate (XL*)

An X-linked recessive form of incomplete clefting of the secondary palate with palatopharyngeal incompetence.

McK 30340

Lowry, R.B. (1970) Sex linked cleft palate in a British Columbia Indian family. *Pediatrics*, **46**, 123–128.

I.B. Disorders in which There Is Often a Cleft of the Lip and/or Palate

I.B.1. (IV.I.3.) Apert syndrome (acrocephalosyndactyly type I) (AD*)

Craniosynostosis producing flattening of the frontal and occipital regions of the skull. There is a high steep forehead, and often ocular hypertelorism, proptosis and antimongoloid obliquity of the palpebral fissures. The middle third of the face is always underdeveloped, resulting in relative prominence of the mandible and, frequently, a class III malocclusion. These features are invariably associated with severe osseous and soft tissue syndactyly of both hands and feet. There may also be aplasia or ankylosis of joints, progressive synostosis of the bones of the hands and feet and of the vertebrae, and retarded tooth eruption. Cleft palate occurs in about one-third of cases.

McK 10120 *OFG* 571, 576–580 *SHN* 32–36

Blank, C.E. (1960) Apert's syndrome (a type of acrocephalosyndactyly): observations on a British series of thirty-nine cases. *Annals of Human Genetics*, **24**, 151–164.
Cohen, M.M. Jr (1975) An etiologic and nosologic overview of craniosynostosis syndromes. *Birth Defects*, **11**(2), 137–189.
Peterson, S.J. & Pruzansky, S. (1974) Palatal anomalies in the syndromes of Apert and Crouzon. *Cleft Palate Journal*, **11**, 394–403.
Solomon, M.P. & Cohen, E.S. (1971) Oral manifestations of acrocephalosyndactyly. Apert's syndrome, a case report. *New York State Dental Journal*, **37**, 421–424.

I.B.2. (IV.A.3., IV.D.4., IV.E.1., IV.I.4.) Cleidocranial dysplasia (cleidocranial dysostosis) (AD*)

See IV.A.3.

I.B.3. (II.A.4.) Congenital lip pits (AD*)

See II.A.4.

I.B.4. Diastrophic dwarfism (AR*)

A combination of severe reduction of the limbs, digital abnormalities, club foot, progressive scoliosis and external ear deformities. Cleft palate is found in over 50 per cent of cases.

McK 22260 *OFG* 586–587 *SHN* 250–252

Taybi, H. (1963) Diastrophic dwarfism. *Radiology*, **80**, 1–10.
Vazquez, A.M. & Lee, F.A. (1968) Diastrophic dwarfism. *Journal of Pediatrics*, **72**, 234–242.
Walker, B.A., Scott, C.I., Hall, J.G., Murdoch, J.L. & McKusick, V.A. (1972) Diastrophic dwarfism. *Medicine*, **51**, 41–60.

I.B.5. Larsen syndrome (multiple congenital dislocations; flattened facies and cleft palate) (H)

A syndrome of flattened facies with a depressed nasal bridge, multiple congenital dislocations, short stature, and digital and foot anomalies. Cleft palate has been observed in about 50 per cent of cases. There appear to be both autosomal dominant and recessive forms of the disorder.

McK 15025 (AD*), 24560 (AR*) *OFG* 544
SHN 128–131

Harris, R. & Cullen, C.H. (1971) Autosomal dominant inheritance in Larsen's syndrome. *Clinical Genetics*, **2**, 87–90.
Latta, R.J., Graham, C.B., Aase, J.M., Scham, S.M. & Smith, D.W. (1971) Larsen's syndrome: a skeletal dysplasia with

multiple joint dislocations and unusual facies. *Journal of Pediatrics*, **78**, 291–298.
Robertson, F.W. (1975) Larsen's syndrome. *Clinical Pediatrics*, **14**, 53–60.
Silverman, F.N. (1972) Larsen's syndrome: congenital dislocation of the knees and other joints, distinctive facies and frequently, cleft palate. *Annals of Radiology*, **15**, 297–328.
Steel, H.H. & Kohl, E.J. (1972) Multiple congenital dislocations associated with other skeletal anomalies (Larsen's syndrome) in three siblings. *Journal of Bone and Joint Surgery*, **54A**, 75–82.

I.B.6. Mandibulofacial dysostosis (*Treacher Collins syndrome*) (AD*)

Characteristic facies, including marked antimongoloid obliquity of the palpebral fissures, malar and mandibular hypoplasia, and deformities of the external ear. The auditory ossicles and cochlear and vestibular apparatus may also be malformed, with consequent deafness. Cleft palate is found in about one-third of cases, and the poor jaw development frequently results in malocclusion. The severity of the disorder is variable, minimally affected individuals sometimes being difficult to distinguish from normal. In some families miscarriage or early postnatal death is common, indicating lethality, whereas in others this is not the case and the disorder may occur over several generations. Malformations appear to be restricted largely to derivatives of the first branchial arch, groove and pouch. This disorder also occurs in conjunction with limb and hand anomalies (*McK* 15440 and 18370).

McK 15450 *OFG* 528–530 *SHN* 453–458

Garner, L.D. (1967) Cephalometric analysis of Berry–Treacher Collins syndrome. *Oral Surgery*, **23**, 320–327.
Huffman, G.G. & Lorson, E.L. (1974) Treatment of malocclusion in a case of Treacher Collins syndrome. *Journal of Oral Surgery*, **32**, 612–616.
Poswillo, D. (1975) The pathogenesis of the Treacher Collins syndrome (mandibulofacial dysostosis). *British Journal of Oral Surgery*, **13**, 1–26.
Rogers, B.O. (1964) Berry–Treacher Collins syndrome. A review of 200 cases. *British Journal of Plastic Surgery*, **17**, 109–137.
Rovin, S., Dachi, S.F., Borenstein, D.B. & Cotter, W.B. (1964) Mandibulofacial dysostosis: a familial study of five generations. *Journal of Pediatrics*, **65**, 215–221.

I.B.7. Meckel syndrome (AR*)

A combination of microcephaly, exencephalocele, microphthalmia, congenital heart defects, polydactyly, and polycystic kidneys, liver and pancreas. Cleft lip and/or palate occurs in about 50 per cent of cases. The syndrome is lethal, death usually occurring soon after birth.

McK 24900 *OFG* 539 *SHN* 465–467

Hsia, Y.E., Bratu, M. & Herbordt, A. (1971) Genetics of the Meckel syndrome (dysencephalia splanchnocystica). *Pediatrics*, **48**, 237–247.

I.B.8. Stickler syndrome (*hereditary arthro-ophthalmopathy with retinal detachment and cleft palate*) (AD*)

A combination of enlargement and hyperextensibility of joints, sometimes painful with use, congenital myopia, and a range of facial abnormalities varying from normality, through midface flattening, to the Robin anomalad (see I.A.17.). There may also be retinal detachment with consequent blindness. The disorder is considered to be a connective tissue dysplasia.

McK 10830 *OFG* 537 *SHN* 149–150

Hall, J. (1974) Stickler syndrome presenting as a syndrome of cleft palate, myopia and blindness inherited as a dominant trait. *Birth Defects*, **10**(8), 157–171.
Say, B., Berry, J. & Barker, N. (1977) The Stickler syndrome (Hereditary arthro-ophthalmopathy). *Clinical Genetics*, **12** 179–182.
Schreiner, R.L., McAlister, W.H., Marshall, R.E. & Shearer, W.T. (1973) Stickler syndrome in a pedigree of Pierre Robin syndrome. *American Journal of Diseases of Children*, **126**, 86–90.

I.C. Disorders Producing Hyperostosis, Osteopetrosis, Osteomas or Odontomas

I.C.1. (*IV.E.3.*) *Gardner syndrome* (*intestinal polyposis III*) (AD*) (see also Chapter 5)

A syndrome of multiple osteomas, especially of the facial bones, epidermoid cysts and fibromas of the skin, and intestinal polyposis. The intestinal polyps frequently undergo malignant degeneration. The osteomas, which appear around puberty and usually precede the intestinal polyposis, are generally described as globoid, the maxilla and mandible being among the bones most frequently affected. There may also be odontomas, more normal unerupted supernumerary teeth, and hypercementosis. Osteosarcoma has been reported in at least one family with intestinal polyposis, but malignancy of the bony lesions does not appear to be usual. Intestinal polyposis also occurs without extraintestinal manifestations (*McK* 17510), and with different oral manifestations (see II.A.10.).

McK 17530 *OFG* 594–595 *SHN* 324–328

Amato, A.E. & Small, E.W. (1970) Oral manifestations of Gardner's syndrome: report of a case. *Journal of Oral Surgery*, **28**, 458–460.
Davies, A.S. (1970) Gardner's syndrome–a case report. *British Journal of Oral Surgery*, **8**, 51–57.
Fader, M., Kline, S.N., Spatz, S.S. & Zubrow, H.J. (1962) Gardner's syndrome (intestinal polyposis, osteomas, sebaceous cysts) and a new dental discovery. *Oral Surgery*, **15**, 153–172.
Hoffman, D.C. & Brooke, B.N. (1970) Familial sarcoma of bone in a polyposis coli family. *Diseases of the Colon and Rectum*, **13**, 119–120.
Neal, C.J. (1969) Multiple osteomas of the mandible associated with polyposis of the colon (Gardner's syndrome). *Oral Surgery*, **28**, 628–631.
Utsunomiya, J. & Nakamura, T. (1975) The occult

osteomatous changes in the mandible in patients with familial polyposis coli. *British Journal of Surgery*, **62**, 45–51.

I.C.2. Generalized cortical hyperostosis (Van Buchem disease: hyperphosphatasia tarda) (AR*)

A disorder characterized by slowly progressive mandibular enlargement and diaphyseal thickening. The bony changes start to occur around puberty, and there may eventually be signs of optic atrophy, deafness, facial paralysis and other cranial nerve involvement, due to the narrowing of cranial foramina with associated nerve compression. The alkaline phosphatase level is increased. The disorder is similar to sclerosteosis (see I.C.9.).

McK 23910 *SHN* 238

Dyson, D.P. (1972) Van Buchem's disease (hyperostosis corticalis generalisata familiaris). A case report. *British Journal of Oral Surgery*, **9**, 237–245.

Van Buchem, F.S.P., Hadders, H.N., Hansen, J.F. & Woldring, M.G. (1962) Hyperostosis corticalis generalisata: report of seven cases. *American Journal of Medicine*, **33**, 387–397.

I.C.3. Infantile cortical hyperostosis (Caffey disease) (AD)

This condition first appears as a tender soft tissue swelling over the affected bones, most frequently the mandible, at between two and four months of age. The swelling may be preceded or accompanied by hyperirritability and mild fever. Radiographic evidence shows that new periosteal bone formation occurs in relation to the soft tissue swelling, but that this resolves, partly or completely, in later life. The disorder bears a superficial resemblance to cherubism, but can be distinguished from it on several grounds (see I.D.3.).

McK 11400 *SHN* 397–400

Ball, M.J. & Feingold, M. (1974) Autosomal dominant inheritance of Caffey's disease. *Birth Defects*, **10**(9), 139–146.

Burbank, P.M., Lovestedt, S.A. & Kennedy, R.L.J. (1958) The dental aspects of infantile cortical hyperostosis. *Oral Surgery*, **11**, 1126–1137.

Caffey, J. (1957) Infantile cortical hyperostosis: review of clinical and radiographic features. *Proceedings of the Royal Society of Medicine*, **50**, 347–354.

Van Buskirk, F.W., Tampas, J.P. & Peterson, O.S. (1961) Infantile cortical hyperostosis: an inquiry into its familial aspects. *American Journal of Roentgenology*, **85**, 613–632.

I.C.4. Juvenile cortical hyperostosis (juvenile Paget disease; congenital hyperphosphatasia) (AR*)

Hyperostosis affecting the skull and long bones, with replacement of the cortical bone by trabecular bone. The disorder appears in early childhood with fever, bone pain, multiple fractures and shedding of teeth. Affected individuals have an enlarged head, short stature and bowed limbs. The alkaline phosphatase level is elevated.

McK 23900 *SHN* 240–241

Caffey, J. (1973) Familial hyperphosphatasemia with ateliosis and hypermetabolism of growing membranous bone: review of the clinical, radiographic and chemical features. In *Intrinsic Diseases of Bones*, Volume 4 of *Progress in Pediatric Radiology* (Ed.) Kaufmann, H.J. pp. 438–468. Basel: S. Karger.

I.C.5. Multiple odontomas, oesophageal stenosis and chronic interstitial cirrhosis of the liver (AD)

Multiple maxillary and mandibular tumours containing large numbers of teeth in various stages of development. The evidence suggests that multiple odontomas can occur either alone or in combination with oesophageal stenosis and cirrhosis of the liver.

McK 16433 *SHN* 751–752

Bader, G. (1967) Odontomatosis (multiple odontomas). *Oral Surgery*, **23**, 770–773.

Browne, W.G. (1970) Familial compound composite odontomes. *Oral Surgery*, **29**, 428–430.

Malik, S.A. & Khalid, M. (1974) Odontomatosis (multiple odontomas)–a case report. *British Journal of Oral Surgery*, **11**, 262–264.

Schmidseder, R. & Hausamen, J.R. (1975) Multiple odontogenic tumors and other anomalies. *Oral Surgery*, **39**, 249–258.

I.C.6. (IV.I.14.) Osteopetrosis ('marble bones'; Albers-Schönberg disease) (H)

A disorder resulting from defective physiological resorption of immature bone, though the formation of new bone is essentially normal. As a consequence, there may be macrocephaly, anaemia due to obliteration of marrow cavities, and progressive deafness and blindness, possibly due to nerve pressure through narrowing of cranial foramina, though there is some evidence for primary retinal atrophy. Other complications include fragility of bones, and dental abscesses that are presumably the result of occlusion of the blood supply to the teeth with consequent pulpal necrosis. There may be fractures and osteomyelitis, particularly of the mandible, and dental eruption may be retarded. The age of onset varies from as early as fetal life to the second decade. There are autosomal dominant and recessive forms, the recessive form generally manifesting itself earlier and being the more severe.

McK 16660 (AD*), 25970 (AR*)

Brown, D.M. & Dent, P.B. (1971) Pathogenesis of osteopetrosis: a comparison of human and animal spectra. *Pediatric Research*, **5**, 181–191.

Enell, H. & Pehrson, M. (1958) Studies on osteopetrosis. I. Clinical report of three cases with genetic considerations. *Acta Paediatrica*, **47**, 279–287.

Johnston, C.C. Jr, Lavy, N., Lord, T., Vellios, F., Merritt, A.D. & Deiss, W.P. Jr (1968) Osteopetrosis. A clinical,

genetic, metabolic and morphologic study of the dominantly inherited benign form. *Medicine*, **47**, 149–167.

I.C.7. (*IV.E.6.*) *Paget disease of bone* (*osteitis deformans*) (AD)

A slowly progressive disorder, usually appearing after the age of 40, characterized by abnormal resorption of bone and excessive apposition of new, abnormally coarse, bone. Deformity results from this excessive apposition and from the bending of weight-bearing bones under stress. Affected bones may be painful and liable to fracture, and have a fluffy 'cotton wool' appearance in radiographs. Other complications include: anaemia, due to invasion of marrow spaces; impaired vision, hearing and other sensory and motor disturbances caused by nerve compression; and cardiac failure, resulting from reduced ventilatory capacity through involvement of the thoracic skeleton, together with unusual vascularity of the bony lesions, demanding an increased cardiac output. In a small proportion of cases osteosarcoma or fibrosarcoma has developed. The maxilla is more frequently involved than the mandible, and may undergo enlargement with consequent distortion of tooth alignment and loosening of the teeth. There may also be hypercementosis. In cases where a denture is worn, it requires replacement often due to the gradual increase in size of the supporting bone. There is a wide variation in severity of the disorder, some cases being asymptomatic and diagnosed only by chance on routine x-ray. The incidence may be as high as 3 per cent among individuals over the age of 40.

McK 16725

Aegerter, E. & Kirkpatrick, J.A. (1975) *Orthopedic Diseases*, pp. 407–419. Philadelphia, London and Toronto: W.B. Saunders.

Cooke, B.E.D. (1956) Paget's disease of the jaws: fifteen cases. *Annals of the Royal College of Surgeons of England*, **19**, 223–240.

Stafne, E.C. & Austin, L.T. (1938) A study of dental roentgenograms in cases of Paget's disease (osteitis deformans), osteitis fibrosa cystica and osteoma. *Journal of the American Dental Association*, **25**, 1202–1214.

I.C.8. (*IV.I.17.*) *Pyknodysostosis* (AR*)

A combination of osteopetrosis, dwarfism, abbreviated terminal phalanges, and various cranial anomalies. There is increased radio-opacity and increased fragility of all bones. The skull shows frontal and occipital bossing and persistence of all sutures and fontanels. The facial bones are underdeveloped and the mandibular angle obtuse. There may be premature or delayed tooth eruption. There is good evidence to suggest that Toulouse-Lautrec had this syndrome.

McK 26580 *OFG* 625–626 *SHN* 639–642

Elmore, S.M. (1967) Pycnodysostosis: a review. *Journal of Bone and Joint Surgery*, **49A**, 153–163.

Maroteaux, P. & Lamy, M. (1965) The malady of Toulouse-Lautrec. *Journal of the American Medical Association*, **191**, 715–717.

I.C.9. Sclerosteosis (*cortical hyperostosis with syndactyly*) (AR*)

A disorder similar to generalized cortical hyperostosis (see I.C.2.), but with an earlier age of onset, a rather different radiographic appearance and frequently, but not always, with soft tissue syndactyly of the index and middle fingers. The mandible has an unusually square appearance when viewed from in front, and there is a high steep forehead, ocular hypertelorism and a broad flat nasal root. These facial features are apparent from infancy. Deafness, facial paralysis and other manifestations of cranial nerve involvement may occur later as a result of nerve compression at narrowed cranial foramina.

McK 26950 *SHN* 238–240

Beighton, P., Davidson, J., Durr, L. & Hamersma, H. (1977) Sclerosteosis. An autosomal recessive disorder. *Clinical Genetics*, **11**, 1–7.

Sugiura, Y. & Yasuhara, T. (1975) Sclerosteosis. *Journal of Bone and Joint Surgery*, **57A**, 273–276.

I.C.10. (*I.D.12., III.B.5., IV.D.19.*) *Tuberous sclerosis* (AD*)

See III.B.5.

I.D. Disorders Producing Radiolucencies

I.D.1. Acro-osteolysis with osteoporosis and changes in skull and mandible (*Cheney syndrome*) (AD*)

A combination of generalized osteoporosis, hypoplasia (rather than dissolution) of the terminal phalanges, abnormal skull shape and short stature. The bones are unusually fragile and liable to fracture, and the joints may be painful. There is almost always early loss of teeth with marked atrophy of the alveolar processes.

McK 10250 *SHN* 28–31

Cheney, W.D. (1965) Acro-osteolysis. *American Journal of Roentgenology*, **94**, 595–607.

Herrmann, J., Zugibe, F.T., Gilbert, E.F. & Opitz, J.M. (1973) Arthro-dento-osteo dysplasia (Hadju-Cheney syndrome). Review of a genetic 'acro-osteolysis' syndrome. *Zeitschrift für Kinderheilkunde*, **114**, 93–110. (Name changed to *European Journal of Pediatrics* since Volume **121**, 1975.)

I.D.2. Basal cell naevus syndrome (*Gorlin syndrome*) (AD*) (see also Chapter 5)

A syndrome of multiple naevoid basal cell carcinomas, cysts of the jaws, vertebral and rib anomalies and abnormal calcifications. The basal cell carcinomas usually appear during adolescence or early adult life and involve particularly the face, neck, back and chest. They are papular lesions, generally lightly pigmented

and sometimes ulcerated, varying from 1 mm to 1 cm in diameter and having a wide range of histological appearances. Not all individuals with the syndrome have the skin lesions. There may be a variety of skeletal anomalies, including bifurcation of the ribs, and rib and vertebral fusions. A number of instances of abnormal calcification of various organs have been described. The intracranial calcifications are the most noteworthy, including bridging of the sella turcica and lamellar calcification of the falx cerebri. Keratocysts of the jaws produce radiolucencies, particularly in the mandibular third molar region. They usually start to appear during adolescence and are the chief cause of complaint in at least half the cases. However, they are difficult to treat, recurrences after removal being common.

McK 10940 *OFG* 596–606 *SHN* 520–526

Hickory, J.E., Gilliland, R.F., Wade, W.M. & Taylor, C.G. (1975) Conservative treatment of cysts of the jaws in nevoid basal cell carcinoma syndrome. Report of a case. *Journal of Oral Surgery*, **33**, 693–697.

Kelley, J.E., Hibbard, E.D. & Giansanti, J.S. (1972) Epidermal nevus syndrome. Report of a case with unusual oral manifestations. *Oral Surgery*, **34**, 774–780.

Millar, A.S., Leifer, C., Pullon, P.A. & Bowser, M.W. (1973) Nevoid basal cell carcinoma syndrome. Report of a pedigree with electron microscopy of skin lesions. *Oral Surgery*, **36**, 533–543.

Rayne, J. (1971) The multiple basal cell naevi syndrome. *British Journal of Oral Surgery*, **9**, 65–71.

Towns, T.M. & Lagattuta, V. (1974) Basal cell nevus syndrome: 20 year follow up. *Journal of Oral Surgery*, **32**, 50–53.

I.D.3. Cherubism (familial multilocular cystic disease of the jaws) (AD*)

A benign self-limited condition characterized by swelling of the lower face, beginning in early childhood and tending to regress around puberty. Expansion of the mandible occurs through the growth of multilocular cystic lesions that contain large numbers of giant cells. The disorder may not always be severe enough for its presence to be suspected on clinical grounds alone. There is a superficial resemblance to infantile cortical hyperostosis (Caffey disease, see I.C.3.). However, in Caffey disease the x-ray appearance of the mandible is quite different, there are usually associated systemic manifestations, onset and resolution occur earlier, and bones other than the mandible are frequently involved also.

McK 11840

Anderson, D.E. & McClendon, J.L. (1962) Cherubism–hereditary fibrous dysplasia of the jaws. I. Genetic considerations. *Oral Surgery*, **15** (Supplement 2), 5–16.

Bixler, D. & Garner, L.D. (1971) Cherubism: a family study to delineate gene action on mandibular growth and development. *Birth Defects*, **7**(7), 222–225.

Jones, W.A. (1965) Cherubism: a thumbnail sketch of its diagnosis and a conservative method of treatment. *Oral Surgery*, **20**, 648–653.

I.D.4. Familial hyperparathyroidism (AD*)

Generalized osteoporosis with consequent reduction of radiodensity, development of a 'ground glass' appearance of bone on x-ray, and loss of the lamina dura around the teeth. There may also be sharply defined round, oval or lobulated areas of particular radiolucency in the jaws, produced by multinucleated giant cell lesions.

McK 14500

Kennett, S. & Pollick, H. (1971) Jaws lesions in familial hyperparathyroidism. *Oral Surgery*, **31**, 502–510.

I.D.5. Gaucher disease (H)

Irregular but clearly circumscribed radiolucent areas in the jaws and other bones, in conjunction with haematological abnormalities, splenomegaly and abnormal yellowish pigmentation of the skin and conjunctiva. Tooth extraction from affected regions of the jaws may result in bleeding complications. Three forms, all autosomal recessive, are recognized. There is an acute infantile form, death usually occurring during the first year; and two adult forms, one of which includes neurological complications. Deficiency of the enzyme glucocerebrosidase is common to all three, so the likelihood is that the different forms are produced by different mutant alleles at the same locus.

MBID 731–746 *McK* 23080 (AR*), 23090 (AR), 23100 (AR) *OFG* 396–397

Bender, I.B. (1959) Dental observations in Gaucher's disease. A twenty year follow up. *Oral Surgery*, **12**, 546–561.

Michanowicz, A.E., Michanowicz, J.P. & Stein, G.M. (1967) Gaucher's disease. Report of a case. *Oral Surgery*, **23**, 36–42.

Sela, J., Polliak, A. & Ulmansky, M. (1972) Involvement of the mandible in Gaucher's disease. *British Journal of Oral Surgery*, **9**, 246–250.

I.D.6. (IV.G.6.) Haemoglobinopathies (H)

Disorders of haemoglobin structure, for example sickle cell anaemia, a haemoglobin beta chain variant. In sickle cell anaemia, there are general signs and symptoms of anaemia together with hyperplasia of the haemopoietic marrow in an attempt to compensate for the poor oxygen-carrying capacity of the blood. In addition to generalized osteoporotic changes there is consequently expansion of medullary spaces with loss of trabeculation in the bones of the jaws. Medullary cavity expansion in the facial bones may be particularly prominent, causing an enlargement of the bones themselves with associated malalignment of the teeth. Microthrombi composed of sickled cells have been demonstrated within the blood vessels of the dental pulp in a patient with sickle cell anaemia who had suffered repeated episodes of tooth pain.

McK 14190 (AD*) *OFG* 424–429

Halstead, C.L. (1970) Oral manifestations of hemoglobino-

pathies. *Oral Surgery*, **30**, 615–623.
Michelson, R.K. & Whitmore, R.B. (1967) Sickle cell anaemia in the dental patient. *Oral Surgery*, **23**, 19–24.
Mourshed, F. & Tuckson, C.R. (1974) A study of the radiographic features of the jaws in sickle cell anemia. *Oral Surgery*, **37**, 812–819.
Parkin, S.F. (1968) Dental treatment for child with thalassemia. *Oral Surgery*, **25**, 12–18.
Ryan, M.D. (1971) Osteomyelitis associated with sickle cell anaemia. *Oral Surgery*, **31**, 754–759.
Witkop, C.J. Jr. (1971) Manifestations of genetic diseases in the human pulp. *Oral Surgery*, **32**, 278–316.

I.D.7. Marfan syndrome (AD*)

A combination of disproportionate skeletal growth with excessive length of the extremities, eye abnormalities (especially subluxation of the lenses), and cardiovascular abnormalities (especially aortic aneurisms). The syndrome is associated with abnormal collagen production. Multiple jaw cysts have been reported, but do not appear to be a frequent finding.

McK 15470 *OFG* 608–610 *SHN* 459–462

Oatis, G.W., Burch, M.S. & Samuels, H.S. (1971) Marfan's syndrome with multiple maxillary and mandibular cysts. Report of a case. *Journal of Oral Surgery*, **29**, 515–519.
Smith, N.H. (1968) Multiple dentigerous cysts associated with arachnodactyly and other skeletal defects. *Oral Surgery*, **25**, 99–107.

I.D.8. (II.A.6., II.B.5., II.C.3., IV.G.3., IV.I.12.) Mucopolysaccharidosis I–H (Hurler syndrome) (AR*)

A syndrome manifesting itself in early infancy, consisting of mental retardation, growth retardation, an enlarged deformed head, coarse and expressionless facial features, progressive corneal clouding, skeletal abnormalities, flexion contractures, hernias and hepatosplenomegaly. Death usually occurs before the age of ten due to pneumonia and cardiac failure. There are increased quantities of intracellular and urinary acid mucopolysaccharides. The tongue is enlarged, and the lips are enlarged and patulous. There may be hypertrophy of the alveolar bone, gingival tissues and adenoids. Tooth eruption tends to be delayed. There are frequently localized areas of bone destruction associated with unerupted teeth, particularly in the mandible, resembling dentigerous cysts. These radiolucent areas are thought to be caused by the accumulation of mucopolysaccharide in hyperplastic dental follicles. Histological and histochemical signs of the disorder have been observed in the dental pulp. The disorder is due to a deficiency of the enzyme alpha-L-iduronidase.

MBID 1282–1307 *McK* 25280 *OFG* 206–207, 303–306, 391–393, 610–614 *SHV* 476–481

Block, C. & Lucatorto, F.M. (1971) Gargoylism: Hurler's syndrome, a case history. *Journal of Oral Medicine*, **26**, 106–112.
Cawson, R.A. (1962) The oral changes in gargoylism. *Proceedings of the Royal Society of Medicine*, **55**, 1066–1070.
Fay, J.T. (1972) An early case of Hurler's syndrome. *Journal of Oral Medicine*, **27**, 64–66.
Gardner, D.G. (1968) Metachromatic cells in the gingiva in Hurler's syndrome. *Oral Surgery*, **26**, 782–789.
Gardner, D.G. (1971) The oral manifestations of Hurler's syndrome. *Oral Surgery*, **32**, 46–57.
Worth, H.M. (1966) Hurler's syndrome: a study of radiologic appearances in the jaws. *Oral Surgery*, **22**, 21–35.

I.D.9. (II.A.7., II.B.6.) Mucopolysaccharidosis II (Hunter syndrome) (XL*)

A similar disorder to mucopolysaccharidosis I–H (Hurler syndrome, see I.D.8.), though generally less severe. There is a type A that has progressive mental retardation, with death usually occurring before the age of 15, and a milder type B compatible with survival and reproduction. The tongue and lips are enlarged, and the same jaw lesions seen in the Hurler syndrome are found. The disorder is due to a deficiency of the enzyme iduronate sulphatase.

MBID 1282–1307 *McK* 30990 *OFG* 393, 612 *SHV* 483–485

Hopkins, R., Watson, J.A., Jones, J.H. & Walker, M. (1973) Two cases of Hunter's syndrome–the anaesthetic and operative difficulties in oral surgery. *British Journal of Oral Surgery*, **10**, 286–299.
Lustmann, J., Bimstein, E. & Yatziv, S. (1975) Dentigerous cysts and radiolucent lesions of the jaw associated with Hunter's syndrome. *Journal of Oral Surgery*, **33**, 679–685.

I.D.10. (III.B.3.) Neurofibromatosis (Von Recklinghausen disease) (AD*)

See III.B.3.

I.D.11. (II.C.1., IV.I.18.) Rutherfurd syndrome (AD*)

See II.C.1.

I.D.12. (I.C.10., III.B.5., IV.D.19.) Tuberous sclerosis (AD*)

See III.B.5.

SINGLE GENE DISORDERS WITH PARTICULAR EFFECTS ON THE LIPS, TONGUE OR GINGIVA

II.A. Disorders Affecting the Lips

II.A.1. Blepharochalasis and 'double lip' (Ascher syndrome) (AD*)

A combination of drooping eyelids and duplication of the upper lip. Occasionally the lower lip is also enlarged. The disorder has been associated with non-toxic enlargement of the thyroid gland.

McK 10990 *SHV* 253–255

Barnett, M.L., Bosshardt, L.L. & Morgan, A.F. (1972) Double lip and double lip with blepharochalasis (Ascher's syndrome). *Oral Surgery*, **34**, 727–733.

Findlay, G.H. (1954) Idiopathic enlargements of the lips: cheilitis granulomatosa, Ascher's syndrome and double lip. *British Journal of Dermatology*, **66**, 129–138.

Papanayatou, P.H. & Hatziotis, J.C. (1973) Ascher's syndrome. *Oral Surgery*, **35**, 467–471.

Swerdloff, G. (1960) Double lip. *Oral Surgery*, **13**, 627–629.

II.A.2. (IV.A.2., IV.D.3., IV.J.1.) Chondroectodermal dysplasia (Ellis–van Creveld syndrome) (AR*)

A syndrome of dwarfism resulting principally from shortening of the distal parts of the extremities, polydactyly, ectodermal dysplasia affecting particularly the nails and teeth, and cardiac malformation. There is invariably fusion of the middle portion of the upper lip to the maxillary gingival margin, so that there is no labial sulcus in the upper incisor region. There is a reduction in number of teeth, and the teeth that do form are usually small and of abnormal morphology. The enamel may be hypoplastic. Natal teeth have been reported.

McK 22550 *OFG* 589–592 *SHV* 80–85

Biggerstaff, R.H. & Mazaheri, M. (1968) Oral manifestations of the Ellis–van Creveld syndrome. *Journal of the American Dental Association*, **77**, 1090–1095.

Winter, G.B. & Geddes, M. (1967) Oral manifestations of chondroectodermal dysplasia (Ellis–van Creveld syndrome). *British Dental Journal*, **122**, 103–107.

II.A.3. (I.A.5.) Cleft lip-palate, mucous cysts of the lower lip, popliteal pterygium, digital and genital anomalies (popliteal pterygium syndrome) (AD*)

See I.A.5.

II.A.4. (I.B.3.) Congenital lip pits (AD*)

Pits or depressions in the vermilion border of the lower lip, usually one on either side of the midline and occasionally located at the tips of slight elevations. Rarely, these elevations may be sufficiently large to fuse in the midline. The pits are 0.5 to 2.5 cm deep, and are usually fistulae communicating with the duct systems of the underlying minor salivary glands. Consequently, they often discharge saliva, either spontaneously or on pressure. About 70 per cent of affected individuals have cleft lip, cleft palate or both. This is an exception to the rule that affected relatives of cleft lip–palate cases have only cleft lip–palate, and that affected relatives of cleft palate cases have only cleft palate. An associated absence of second premolars has been reported. The gene has an estimated penetrance of 80 per cent.

McK 11930 *OFG* 556–557 *SHV* 115–117

Baker, B.R. (1964) A family with bilateral congenital pits of the inferior lip. *Oral Surgery*, **18**, 494–497.

Bowers, D.G. (1970) Congenital lower lip sinuses with cleft palate. *Plastic and Reconstructive Surgery*, **45**, 151–154.

Červenka, J., Gorlin, R.J. & Anderson, V.E. (1967) The syndrome of pits of the lower lip and cleft lip and/or palate: genetic considerations. *American Journal of Human Genetics*, **19**, 416–432.

Schneider, E.L. (1973) Lip pits and congenital absence of second premolars: varied expression of the lip pits syndrome. *Journal of Medical Genetics*, **10**, 346–349.

II.A.5. Craniocarpotarsal dysplasia (whistling face syndrome; Freeman–Sheldon syndrome) (AD*)

A combination of microstomia, flat midface, deeply sunken eyes, contractures of the fingers, and club foot. The lips are pursed as in whistling. Two grooves extend over the surface of the skin from the lower lip to the chin, one on either side of a central fibrous band.

McK 19370 *OFG* 567–569 *SHV* 214–219

Burzynski, N.J., Podruch, P.E., Howell, J. & Snawder, K. (1975) Craniocarpotarsal dysplasia (whistling face syndrome). *Oral Surgery*, **39**, 893–900.

Červenka, J., Figalova, P. & Gorlin, R.J. (1969) Cranio-carpo-tarsal dysplasia or the whistling face syndrome. II. Oral intercommissural distance in children. *American Journal of Diseases of Children*, **117**, 434–435.

Sauk, J.J. Jr, Delaney, J.R., Reaume, C., Brandjord, R. & Witkop, C.J. (1974) Electromyography of oral-facial musculature in craniocarpotarsal dysplasia (Freeman-Sheldon syndrome). *Clinical Genetics*, **6**, 132–137.

Weinstein, S. & Gorlin, R.J. (1969) Cranio-carpo-tarsal dysplasia or the whistling face syndrome. I. Clinical considerations. *American Journal of Diseases of Children*, **117**, 427–433.

II.A.6. (I.D.8., II.B.5., II.C.3., IV.G.3., IV.I.12.) Mucopolysaccharidosis I–H (Hurler syndrome) (AR*)

See I.D.8.

***II.A.7.* (*I.D.9., II.B.6.*) *Mucopolysaccharidosis II* (*Hunter syndrome*)** (XL*)

See I.D.9.

***II.A.8.* (*I.A.13., II.B.7., IV.A.14.*) *Oral–facial–digital syndrome I* (*OFD I syndrome*)** (XL*)

See I.A.13.

***II.A.9.* (*II.B.8.*) *Oral–facial–digital syndrome II* (*OFD II syndrome; Mohr syndrome*)** (AR*)

See II.B.8.

***II.A.10.* (*III.C.5.*) *Peutz–Jeghers syndrome* (*intestinal polyposis II*)** (AD*) (see also Chapter 5)

A combination of gastrointestinal polyposis and mucocutaneous melanotic pigmentation. Onset of the gastrointestinal symptoms can occur at any age, but the polyps rarely undergo malignant change. Almost all affected individuals show melanotic pigmentation of the lips, brown to bluish-black macules ranging in size from 1 mm to 12 mm across but of no particular shape. The oral mucosa may be involved, though slightly less often, and the skin, particularly around the mouth, nose and eyes, is affected in about 50 per cent of cases. Pigmented oral papillomas have also been reported. Intestinal polyposis can occur without oral manifestations (*McK* 17510) or with different oral manifestations (see I.C.1.).

McK 17520 *OFG* 359–360 *SHV* 604–608

Bartholomew, L.G., Moore, C., Dahlin, D.C. & Waugh, J.M. (1962) Intestinal polyposis associated with mucocutaneous pigmentation. *Surgery, Gynecology and Obstetrics*, **115**, 1–11.

Lowe, N.J. (1975) Peutz-Jeghers syndrome with pigmented oral papillomas. *Archives of Dermatology*, **111**, 503–505.

Zegarelli, E.V., Kesten, B.M. & Kutscher, A.H. (1954) Melanin spots of the oral mucosa and skin associated with polyps: report of a case of peculiar pigmentation of the lips and mouth. *Oral Surgery*, **7**, 972–978.

Zegarelli, E.V., Kutscher, A.H., Mercadante, J., Kupferberg, N. & Piro, J.D. (1959) Atlas of oral lesions observed in the syndrome of oral melanosis with associated intestinal polyposis (Peutz-Jeghers syndrome). *American Journal of Digestive Diseases*, N.S. **4**, 479–489.

II.B. Disorders Affecting the Tongue

***II.B.1. Beckwith–Wiedemann syndrome* (*exomphalos–macroglossia–gigantism syndrome*)** (AR)

A syndrome of umbilical hernia, cytomegaly of the adrenal cortex, hyperplasia of the gonadal interstitial cells, renal medullary dysplasia, visceromegaly, somatic gigantism, mild microcephaly, and a dome-shaped defect of the diaphragm. Macroglossia is very common, but not an essential feature.

McK 22560 *OFG* 526–528 *SHV* 42–47

Cohen, M.M. Jr, Gorlin, R.J., Feingold, M. & Bensei, R.W. (1971) The Beckwith-Wiedemann syndrome: seven new cases. *American Journal of Diseases of Children*, **122**, 515–519.

Filippi, G. & McKusick, V.A. (1970) Beckwith Wiedemann syndrome (exomphalos-macroglossia-gigantism syndrome). Report of two cases and review of the literature. *Medicine*, **49**, 279–298.

Kosseff, A.L., Hermann, J. & Opitz, J.M. (1972) The Wiedemann-Beckwith syndrome: genetic considerations and a diagnostic sign. *Lancet*, **i**, 844.

Thorburn, M.J., Wright, E.S., Miller, C.G. & Smith-Read, E.H.M. (1970) Exomphalos-macroglossia-gigantism syndrome in Jamaican infants. *American Journal of Diseases of Children*, **119**, 316–321.

***II.B.2.* (*V.B.1.*) *Familial dysautonomia* (*Riley–Day syndrome*)** (AR*)

See V.B.1.

***II.B.3.* (*V.B.4.*) *Melkersson syndrome* (*Melkersson–Rosenthal syndrome*)** (AD*)

See V.B.4.

***II.B.4.* (*V.B.5.*) *Moebius syndrome* (*congenital facial diplegia*)** (AD*)

See V.B.5.

***II.B.5.* (*I.D.8., II.A.6., II.C.3., IV.G.3., IV.I.12.*) *Mucopolysaccharidosis I–H* (*Hurler syndrome*)** (AR*)

See I.D.8.

***II.B.6.* (*I.D.9., II.A.7.*) *Mucopolysaccharidosis II* (*Hunter syndrome*)** (XL*)

See I.D.9.

***II.B.7.* (*I.A.13., II.A.8., IV.A.14.*) *Oral–facial–digital syndrome I* (*OFD I syndrome*)** (XL*)

See I.A.13.

***II.B.8.* (*II.A.9.*) *Oral–facial–digital syndrome II* (*OFD II syndrome; Mohr syndrome*)** (AR*)

A syndrome of cleft or lobed tongue, manual polydactyly, polysyndactyly of the halluces, and frequently a midline cleft of the upper lip. Mental deficiency,

microcephaly, hydrocephaly, deafness, various other neurological abnormalities, and increased susceptibility to respiratory infection have also been reported. Occasional oral findings include multiple frenula, ankyloglossia and fatty hamartomas of the dorsum of the tongue.

McK 25210 *OFG* 560–561 *SHN* 568–570

Goldstein, E. & Medina, J.L. (1974) Mohr syndrome or oral-facial-digital II: report of two cases. *Journal of the American Dental Association*, **89**, 377–382.

Gustavson, K.H., Kreuger, A. & Petersson, P.O. (1971) Syndrome characterised by lingual malformation, polydactyly, tachypnea, and psychomotor retardation (Mohr syndrome). *Clinical Genetics*, **2**, 261–266.

Rimoin, D.L. & Edgerton, M.T. (1967) Genetic and clinical heterogeneity in the oral-facial-digital syndromes. *Journal of Pediatrics*, **71**, 94–102.

II.C. Disorders Affecting the Gingiva

II.C.1. Gingival fibromatosis (H)

Gingival fibromatosis may occur either alone or in conjunction with a variety of other abnormalities. General references are:

OFG 596 *SHN* 329–336

Witkop, C.J. Jr (1971) Heterogeneity in gingival fibromatosis. *Birth Defects*, **7**(7), 210–221.

A list of disorders in which gingival fibromatosis occurs is given below:

Gingival fibromatosis without hypertrichosis (AD). Gingival fibromatosis unassociated with any other abnormality. However, cases of supposed isolated gingival fibromatosis may have had minimal hypertrichosis, that is, 'without hypertrichosis' and 'with hypertrichosis' cases (see below) may represent different levels of manifestation of the same mutant gene.

McK 13530

Becker, W., Collings, C.K., Zimmerman, E.R., De La Rosa, M. & Singdahlsen, D. (1967) Hereditary gingival fibromatosis. *Oral Surgery*, **24**, 313–318.

Zackin, S.J. & Weisberger, D. (1961) Hereditary gingival fibromatosis. Report of a family. *Oral Surgery*, **14**, 828–836.

Gingival fibromatosis with hypertrichosis (AD*). Gingival fibromatosis associated with hypertrichosis alone or with hypertrichosis, mental deficiency and/or epilepsy.

McK 13540

Jorgensen, R.J. & Cocker, M.E. (1974) Variation in the inheritance and expression of gingival fibromatosis. *Journal of Periodontology*, **45**, 472–477.

Miles, A.E.W. (1974) Julia Pastrana, the Bearded Lady. *Proceedings of the Royal Society of Medicine*, **67**, 160–164.

Nevin, N.C., Scally, B.G., Kernohan, D.C. & Dodge, J.A. (1971) Hereditary gingival fibromatosis. *Journal of Mental Deficiency Research*, **15**, 130–135.

Snyder, C.H. (1965) Syndrome of gingival hyperplasia, hirsutism and convulsions. *Journal of Pediatrics*, **67**, 499–502.

Winter, G.B. & Simpkiss, M.J. (1974) Hypertrichosis with hereditary gingival hyperplasia. *Archives of Disease in Childhood*, **49**, 349–399.

Gingival fibromatosis with ear, nose, bone, nail defects and splenomegaly (Laband syndrome) (AD*). In this syndrome cartilage has a soft consistency, resulting in poor structural definition of the nose and external ear. Fingers and toes may lack terminal phalanges, nails are hypoplastic or even absent, and the liver and spleen are enlarged. Gingival fibromatosis has been observed in every case.

McK 13550

Alvandar, G. (1965) Elephantiasis gingivae. Report of an affected family with associated hepatomegaly, soft tissue and skeletal abnormalities. *Journal of the All India Dental Association*, **37**, 349–353.

Leband, P.F., Habib, G. & Humphreys, G.S. (1964) Hereditary gingival fibromatosis. Report of an affected family with associated splenomegaly and skeletal and soft tissue abnormalities. *Oral Surgery*, **17**, 339–351.

Gingival fibromatosis with microphthalmia, mental retardation, athetosis and hypopigmentation (Cross syndrome, oculo-cerebral syndrome with hypopigmentation) (AR*). Gingival fibromatosis in conjunction with microphthalmia, cloudy cornea, spasticity, mental retardation, athetoid movements and hypopigmentation.

McK 25780

Cross, H.E., McKusick, V.A. & Breen, W.A. (1967) A new oculocerebral syndrome with hypopigmentation. *Journal of Pediatrics*, **70**, 398–406.

Gingival fibromatosis with progressive deafness (AD). Gingival fibromatosis associated with progressive hearing loss that becomes symptomatic late in the second decade of life.

McK 13555

Jones, G., Willroy, R.S. & McHaney, V. (1977) Familial gingival fibromatosis associated with progressive deafness in five generations of a family. *Birth Defects*, **13**(3B), 195–201.

Juvenile hyaline fibromatosis (Murray–Puretić–Drescher syndrome) (AR*). Multiple hyaline fibrous tumours, especially of the scalp and back, associated with gingival fibromatosis. The tumours appear in early life, slowly enlarging to the size of small oranges. Some gradually regress, while others calcify, ulcerate and slowly disappear. There may be painful flexion contractures of the knees, elbows, hips and shoulders, generalized osteoporosis, osteolysis of the terminal phalanges, atrophic changes in the skin and recurrent suppurative infections. There are two entries in McKusick's catalogue: juvenile fibromatosis (22860) and Puretić syndrome (26570).

McK 22860, 26570

Drescher, E., Woyke, S., Markiewicz, C. & Tegi, S. (1967) Juvenile fibromatosis in siblings (fibromatosis hyalinica multiplex juvenilis). *Journal of Pediatric Surgery*, **2**, 427–430.

Ishikawa, H. & Mori, S. (1973) Systemic hyalinosis or

fibromatosis multiplex juvenilis as a congenital syndrome. *Acta Dermato-Venereologica (Stockholm)*, **53**, 185–191.

Puretić, S. & Puretić, B. (1971) Clinical and histopathological observations on systemic familial mesenchymatosis. *Proceedings of the 13th International Congress on Pediatrics, Vienna, 1971*, **5**, 373–381.

Rutherfurd syndrome (AD*). Mild gingival fibromatosis associated with mental deficiency, aggressive behaviour, corneal opacities, failure of tooth eruption, root resorption and dentigerous cysts. (Also listed under I.D.11 and IV.I.18.)

McK 18090

Houston, I.B. & Shotts, N. (1966) Rutherfurd's syndrome: a familial oculodental disorder. *Acta Paediatrica Scandinavica*, **55**, 233–238.

Rutherfurd, M.E. (1931) Three generations of inherited dental defect. *British Medical Journal*, **ii**, 9–11.

II.C.2. (*IV.D.14.*) *Mucolipidosis II* (*I-cell disease*) (AR*)

A combination of severe psychomotor retardation, marked shortness of stature, Hurler-like facial features (see I.D.8.) and impressive gingival enlargement. Death usually occurs in childhood as a result of cardiac failure. There is marked hypocalcification of the dental enamel. The disorder is known as I-cell disease because of numerous granular inclusions visible in fibroblast cytoplasm under phase contrast microscopy.

McK 25250 *OFG* 394 *SHN* 497–499

Blank, E. & Linder, D. (1974) I-cell disease (ML II): a lysosomopathy. *Pediatrics*, **54**, 797–805.

Galili, D., Yatziv, S. & Russell, A. (1974) Massive gingival hyperplasia preceding dental eruption in I-cell disease. *Oral Surgery*, **37**, 533–539.

Leroy, J.G., Spranger, J.W., Feingold, M., Opitz, J.M. & Crocker, A.C. (1971) I-cell disease: a clinical picture. *Journal of Pediatrics*, **79**, 360–365.

II.C.3. (*I.D.8., II.A.6., II.B.5., IV.G.3., IV.I.12.*) *Mucopolysaccharidosis I–H* (*Hurler syndrome*) (AR*)

See I.D.8.

II.C.4. Palmoplantar hyperkeratosis and attached gingival hyperkeratosis (AD)

Hyperkeratosis of the palms and soles associated with sharply demarcated hyperkeratosis of the labial and lingual attached gingiva, appearing in early childhood and increasing in severity with age.

McK 14855 *OFG* 367–369 *SHN* 371–372

Fred, H.L., Gieser, R.G., Berry, W.R. & Eiband, J.M. (1964) Keratosis palmaris et plantaris. *Archives of Internal Medicine*, **113**, 866–871.

James, P. & Beggs, D. (1973) Tylosis: a case report. *British Journal of Oral Surgery*, **11**, 143–145.

Raphael, A.L., Baer, P.N. & Lee, W.B. (1968) Hyperkeratosis of gingival and plantar surfaces. *Periodontics*, **6**, 118–120.

SINGLE GENE DISORDERS AFFECTING THE ORAL MUCOSA AND UNDERLYING SOFT TISSUES

III.A. Disorders Resulting in Mucosal Thickening or Abnormal Keratinization

III.A.1. (*III.C.1.*) *Acanthosis nigricans* (AD*) (see also Chapters 5 and 12)

A condition with a severe non-heritable form, associated with adenocarcinoma, particularly of the stomach, and a benign form showing dominant inheritance. Thickening, pigmentation and papillomatous changes develop in the skin, particularly on flexural surfaces. There may also be thickening and papillomatosis of the oral mucosa, particularly of the tongue and lips, and gingival enlargement.

McK 10060 *SHN* 4–8

Cohenour, W. & Gamble, J.W. (1971) Acanthosis nigricans: review of literature and report of a case. *Journal of Oral Surgery*, **29**, 48–51.

Pindborg, J.J. & Gorlin, R.J. (1962) Oral changes in acanthosis nigricans: juvenile type. *Acta Dermato-Venereologica (Stockholm)*, **42**, 63–71.

III.A.2. (*IV.I.7.*) *Cutis laxa* (H)

Generalized laxity of the skin producing a 'bloodhound' or elderly appearance in the face. There may be oral mucosal thickening and delayed or disturbed tooth eruption. There are autosomal dominant, autosomal recessive and X-linked forms. The X-linked form has been associated with deficiency of the enzyme lysyl oxidase.

McK 12370 (AD*), 21910 (AR*), 30415 (XL*)
SHN 246–249

Beighton, P.H. (1972) The dominant and recessive forms of cutis laxa. *Journal of Medical Genetics*, **9**, 216–221.

Goltz, R.W., Hult, A.M., Goldfarb, M. & Gorlin, R.J. (1965) Cutix laxa, a manifestation of generalized elastosis. *Archives of Dermatology*, **92**, 373–387.

III.A.3. Darier–White disease (*Darier disease; follicular keratosis*) (AD*)

A disorder characterized by cutaneous hyperkeratotic papules, 2 to 3 mm across, first appearing in childhood

and changing from skin colour to greyish-brown with age. Similar hyperkeratotic papules occur on the oral mucosa, particularly of the hard and soft palates, and have the whitish appearance common to hyperkeratotic lesions in the mouth. The papules may become confluent as the disorder progresses.

McK 12420 *OFG* 365–366

Gorlin, R.J. (1969) Genetic disorders affecting the mucous membranes. *Oral Surgery*, **28**, 512–525.
Gorlin, R.J. & Chaudhry, A.P. (1959) Oral manifestations of keratosis follicularis. *Oral Surgery*, **12**, 1468–1470.
Rayne, J. (1970) Keratosis follicularis. A case report. *British Journal of Oral Surgery*, **8**, 133–137.
Spouge, J.D., Trott, J.R. & Chesko, G. (1966) Darier-White's disease: a cause of white lesions of the mucosa. *Oral Surgery*, **21**, 441–457.
Weathers, D.R. & Driscoll, R.M. (1974) Darier's disease of the oral mucosa. *Oral Surgery*, **37**, 711–721.

III.A.4. Hereditary benign intraepithelial dyskeratosis (AD*)

Asymptomatic, soft white plaques on the oral mucosa, sometimes folded and similar in appearance to the white sponge naevus of Cannon (see III.A.7.), usually apparent at birth and increasing in severity with age. There are associated eye lesions, and, if these involve the cornea, blindness may result. Buccal scraping smears can be used as an aid to diagnosis, showing the 'cell-within-a-cell' phenomenon.

McK 12760 *OFG* 360–363 *SHV* 349–351

Pollitzer, W.S., Menegaz-Bock, R.M., Renwick, J.H. & Witkop, C.J. (1965) Hereditary benign intraepithelial dyskeratosis: a linkage study. *American Journal of Human Genetics*, **17**, 104–108.
Witkop, C.J. & Gorlin, R.J. (1961) Four hereditary mucosal syndromes. *Archives of Dermatology*, **84**, 762–771.
Witkop, C.J., Shankle, C.M., Graham, J.B., Murray, M.R., Rucknagel, D.L., & Byerly, B.H. (1960) Hereditary benign intraepithelial dyskeratosis. II. Oral manifestations and hereditary transmission. *Archives of Pathology*, **70**, 696–711.
Yanoff, M. (1968) Hereditary benign intraepithelial dyskeratosis. *Archives of Ophthalmology*, **79**, 291–293.

III.A.5. (IV.J.4.) Pachyonychia congenita (AD*)

There are two forms of this disorder:

Jadassohn–Lewandowski syndrome. Thickening of the nails at or soon after birth, sometimes with palmoplantar hyperkeratosis and hyperhidrosis. There is a general thickening of the skin and oral mucosa due to acanthosis and parakeratosis. This may produce white lesions of the oral mucosa. The dorsum of the tongue is usually the most severely affected area, showing marked thickening and having a greyish-white appearance. Aphthous ulceration is common.

Jackson–Lawler syndrome. As above, but with no oral lesions. In addition, there may be epidermoid cysts and natal teeth.

McK 16720 *OFG* 364–365 *SHV* 600–603

Gorlin, R.J. & Chaudhry, A.P. (1958) Oral lesions accompanying pachyonychia congenita. *Oral Surgery*, **11**, 541–544.
Shrank, A.B. (1966) Pachyonychia congenita. *Proceedings of the Royal Society of Medicine*, **59**, 975–976.
Soderquist, N.A. & Reed, W.B. (1968) Pachyonychia congenita with epidermal cysts and other congenital dyskeratoses. *Archives of Dermatology*, **97**, 31–33.
Young, L.L. & Lennox, J.A. (1973) Pachyonychia congenita. A long term evaluation of associated oral and dermal lesions. *Oral Surgery*, **36**, 663–666.

III.A.6. Porokeratosis (Mibelli disease) (AD*)

Chronic, slow-growing, raised, ring-like keratotic lesions of the skin with atrophic centres, appearing in early life, especially on the hands and feet. There may be similar oral lesions, particularly on the upper lip.

McK 17580 *OFG* 372

Ramanathan, K., Omar-Ahmad, U.D., Kutty, M.K., Ching, L.K. & Dutt, A.K. (1969) Porokeratosis Mibelli. *British Dental Journal*, **126**, 31–32.
Reed, R.J. & Leone, P. (1970) Porokeratosis–a mutant clonal keratosis of the epidermis. Histogenesis. *Archives of Dermatology*, **101**, 340–347.

III.A.7. White sponge naevus of Cannon (white folded dysplasia of the mucous membranes) (AD*)

A thickened, spongy, folded lesion of the oral mucosa with an opalescent white appearance. It is either congenital or appears before puberty on any part of the oral mucosa. There is hyperkeratosis, with intracellular oedema and pyknosis of the cells in the stratum spinosum. The condition is differentiated from hereditary benign intraepithelial dyskeratosis (see III.A.4.) by associated vaginal and anal involvement, and by the absence of conjunctival lesions and the 'cell-within-a-cell' phenomenon.

McK 19390 *OFG* 360

Browne, W.G., Izatt, M.M. & Renwick, J.H. (1969) White sponge naevus of the mucosa: clinical and linkage data. *Annals of Human Genetics*, **32**, 271–281.
McGinnis, J.P. & Turner, J.E. (1975) Ultrastructure of the white sponge naevus. *Oral Surgery*, **40**, 644–651.
Simpson, H.E. (1966) White sponge naevus. *Journal of Oral Surgery*, **24**, 463–466.
Stiff, R.H. & Ferraro, E. (1969) Hereditary keratosis. *Oral Surgery*, **28**, 697–701.
Witkop, C.J. & Gorlin, R.J. (1961) Four hereditary mucosal syndromes. *Archives of Dermatology*, **84**, 762–771.

III.B. Disorders Resulting in Nodular Infiltration, Fibrosis, or Deeper Soft Tissue Lesions

III.B.1. (IV.A.13., IV.D.13.) Lipoid proteinosis (hyalinosis cutis et mucosae) (AR*)

Discrete or confluent yellowish nodules in the skin, 1 to

3 mm across, usually appearing in early life, particularly on the face and hands. There may be wart-like hyperkeratotic lesions on the knees and elbows, and bead-like excrescences along the margins of the eyelids with loss of the lashes. Laryngeal involvement produces hoarseness, and intracranial calcification may result in neurological manifestations. Nearly all the oral soft tissues become infiltrated by raised, yellowish, pea-sized plaques, usually appearing before puberty and increasing in severity with age. The tongue may become firm and woody and lose its papillae. Ulcers may occur. Buccal mucosal involvement may cause stenosis of the parotid duct, with consequent parotitis. Teeth may fail to develop or the enamel may be hypoplastic.

McK 24710 *OFG* 373–375 *SHN* 366–370

Orton, C.I. & Buchan, N.G. (1975) Lipoid proteinosis–the orofacial manifestations. *British Journal of Oral Surgery*, **12**, 289–291.

Simpson, H.E. (1972) Oral manifestations of lipoid proteinosis. *Oral Surgery*, **33**, 528–532.

Williams, R.F. (1971) Lipoid proteinosis. *Oral Surgery*, **31**, 624–626.

III.B.2. Multiple mucosal neuromas, phaeochromocytoma and medullary thyroid carcinoma (AD*) (see also Chapter 5)

A syndrome of multiple mucosal neuromas, tumours of the chromaffin tissue of the adrenal medulla (phaeochromocytomas) and carcinoma of the thyroid medulla. Phaeochromocytomas are associated with hypertension, headache, weakness and flushing. Medullary thyroid carcinoma results in overproduction of calcitonin, serotonin and various other substances. Body build is often reminiscent of the Marfan syndrome (see I.D.7.). Mucosal neuromas are particularly common in the lips, where they lead to nodular enlargement, and on the tongue. The buccal mucosa is involved less frequently. Neuromas may also arise from the conjunctiva, and from the nasal and laryngeal mucosa.

Mck 16230 *OFG* 356–358 *SHN* 513–519

Bartlett, R.C., Myall, R.W.T., Bean, L.R. & Mandelstam, P. (1971) A neuropolyendocrine syndrome: mucosal neuromas, pheochromocytoma and medullary thyroid carcinoma. *Oral Surgery*, **31**, 206–220.

Carney, J.A., Sizemore, G.W. & Lovestedt, S.A. (1976) Mucosal ganglioneuromatosis, medullary thyroid carcinoma and pheochromocytoma: multiple endocrine neoplasia, type 2b. *Oral Surgery*, **41**, 739–752.

Miller, R.L., Burzynski, N.J. & Giammara, B.L. (1977) The ultrastructure of oral neuromas in multiple mucosal neuromas, pheochromocytoma, medullary thyroid carcinoma syndrome. *Oral Pathology*, **6**, 253–263.

Simpson, H.E. (1965) Oral neurofibromatosis with differentiation of sensory end organs. *Oral Surgery*, **19**, 228–233.

Walker, D.M. (1973) Oral mucosal neuroma–medullary thyroid carcinoma syndrome. *British Journal of Dermatology*, **88**, 599–603.

III.B.3. (I.D.10.) Neurofibromatosis (Von Recklinghausen disease) (AD*) (see also Chapter 5)

A syndrome of multiple neurofibromas, cutaneous pigmentation in the form of café-au-lait spots, and skeletal abnormalities including kyphoscoliosis and pseudoarthroses, sometimes with central nervous system involvement. There is a tendency for the tumours to undergo malignant change. Neurofibromas are particularly common in the skin, but may occur in any part of the body. Oral neurofibromas have been reported in less than 10 per cent of cases, but may involve any of the oral soft tissues. Extrabony or intrabony tumours may result in subperiosteal erosive changes or central radiolucencies in the maxilla and mandible. There is a wide range of severity of the disorder.

McK 16220 *OFG* 615–618 *SHN* 535–540

Freedus, M.S. & Doyle, P.K. (1975) Multiple neurofibromatosis with oral manifestations. *Journal of Oral Surgery*, **33**, 360–363.

Freeman, M.J. & Standish, S.M. (1965) Facial and oral manifestations of familial disseminated neurofibromatosis. *Oral Surgery*, **19**, 52–59.

Koblin, I. & Reil, B. (1975) Changes in the facial skeleton in cases of neurofibromatosis. *Journal of Maxillofacial Surgery*, **3**, 23–27.

Rittersma, J., Ten Kate, L.P. & Westerink, P. (1972) Neurofibromatosis with mandibular deformities. *Oral Surgery*, **33**, 718–727.

III.B.4. Pseudoxanthoma elasticum (H)

A combination of skin changes, recurrent severe gastrointestinal bleeding, retinal haemorrhages with consequent failing vision, and weak peripheral pulses. The primary abnormality is calcification of elastic connective tissue fibres. In the skin this produces raised, flat, yellowish papules, especially on flexural surfaces, usually appearing in early adult life. The thickened, yellowish skin eventually becomes loose, folded, and apparently redundant. Haemorrhage occurs because of the involvement of small blood vessels, and the weak pulse is due to calcification of peripheral arteries. About 10 per cent of cases have intramucosal nodules in the mouth, especially in the lower lip. There are autosomal dominant and autosomal recessive forms of the disorder.

McK 17785 (AD*), 26480 (AR*)
OFG 372–373 *SHN* 634–638

Danielsen, L., Kobayasi, T. & Larsen, H.W. (1970) Pseudoxanthoma elasticum, a clinico-pathological study. *Acta Dermato-Venereologica (Stockholm)*, **50**, 355–373.

Pope, F.M. (1974) Autosomal dominant pseudoxanthoma elasticum. *Journal of Medical Genetics*, **11**, 152–157.

Pope, F.M. (1974) Two types of autosomal recessive pseudoxanthoma elasticum. *Archives of Dermatology*, **110**, 209–212.

III.B.5. (*I.C.10., I.D.12., IV.D.19.*) *Tuberous sclerosis* (AD*)

A triad of epilepsy, mental retardation and cutaneous angiofibromas, the first signs usually appearing in early childhood. The most common skin lesions take the form of clusters, sometimes massive, of reddish, flat or rounded growths of various sizes, particularly on and around the nose, and on the cheeks and chin. There may also be fibromatous masses in the skin of the back and scalp and along the nail beds, and patchy loss of pigmentation of the skin. The epilepsy and mental retardation are due to the growth of potato-like (tuberous) masses of glial elements within the brain. There may also be retinal tumours. Fibrous outgrowths of the oral mucosa, particularly from the anterior gingiva, have been noted in about 11 per cent of cases of one series. Cystic radiolucencies and hyperostosis of the jaws have been reported. Hypoplastic enamel defects are also found.

McK 19110 *OFG* 198–200, 634–636 *SHN* 704–708

Davis, R.K., Baer, P.N., Archard, H.O. & Palmer, J.H. (1964) Tuberous sclerosis with oral manifestations. *Oral Surgery*, **17**, 395–400.

Hoff, M., van Grunsven, M.F., Jongebloed, W.L. & 's-Gravenmade, E.J. (1975) Enamel defects associated with tuberous sclerosis: a clinical and scanning-electron-microscope study. *Oral Surgery*, **40**, 261–269.

Papanayotou, P. & Vezirtzi, E. (1975) Tuberous sclerosis with gingival lesions. *Oral Surgery*, **39**, 578–582.

Rushton, M.A. (1956) Some less common bone lesions affecting the jaws. *Oral Surgery*, **9**, 284–304.

Wedgewood, D. & Skene-Smith, H.S. (1973) Tuberose sclerosis with unilateral involvement of the maxilla and mandible: a case report. *British Journal of Oral Surgery*, **11**, 126–130.

III.B.6. Xanthomatoses (H)

Conditions in which there is an excess of lipids in the body due to disturbances of lipid metabolism. Yellowish nodules (xanthomas) containing lipid deposits develop in the skin. Oral xanthomas have been associated with two autosomal recessive disorders of lipid metabolism, familial hyperprebetalipoproteinaemia and familial hyperchylomicronaemia, the second of which is associated with low levels of activity of the enzyme lipoprotein lipase.

MBID 656–669 *McK* 23840 (AR), 23860 (AR*)
OFG 376–377

Raffle, E.J. & Hall, D.C. (1968) Xanthomatosis presenting with oral lesions. *British Dental Journal*, **125**, 62–66.

III.C. Disorders Resulting in Papillomatosis

III.C.1. (*III.A.1.*) *Acanthosis nigricans* (AD*)

See III.A.1.

III.C.2. (*III.D.1., III.H.1.*) *Acrodermatitis enteropathica* (AR*)

See III.D.1.

III.C.3. (*IV.A.6., IV.D.9., IV.I.9.*) *Focal dermal hypoplasia* (*Goltz syndrome*) (XL*)

Multiple papillomas of the mucous membranes, associated with atrophy and streaky hyperpigmentation of the skin, localized deposits of superficial fat, syndactyly and dystrophic nails. There may be hypodontia, enamel fragility and delayed tooth eruption. A variety of other abnormalities have been reported. The disorder is inherited as an X-linked dominant condition, limited to females and apparently lethal in males.

McK 30560 *SHN* 310–314

Daly, J.G. (1968) Focal dermal hypoplasia. *Cutis*, **4**, 1354–1359.

Ferrara, A. (1972) Goltz's syndrome. *American Journal of Diseases of Children*, **123**, 263.

Goltz, R.W., Henderson, R.R., Hitch, J.M. & Ott, J.E. (1970) Focal dermal hypoplasia syndrome. A review of the literature and report of two cases. *Archives of Dermatology*, **101**, 1–11.

Ishibashi, A. & Kurihara, Y. (1972) Goltz's syndrome: focal dermal hypoplasia syndrome. *Dermatologica*, **144**, 156–167.

Ruiz-Maldonado, R., Carnevale, A., Tamayo, L. & de Montiel, E.M. (1974) Focal dermal hypoplasia. *Clinical Genetics*, **6**, 36–45.

III.C.4. Multiple hamartoma and neoplasia syndrome (*Cowden syndrome*) (AD*)

A syndrome of multiple tumours in which there may be papillomatous outgrowths from the buccal mucosa and papular lesions of the lips, gingiva and palate. The tumours include fibroadenomas and carcinomas of the breast, adenocarcinomas of the thyroid, and colonic polyposis and carcinomas. Large numbers of small papillomatous lesions occur on the skin, particularly of the external ear, neck and nose.

McK 15835 *OFG* 521 *SHN* 510–512

Gentry, W.C. Jr, Eskritt, N.R. & Gorlin, R.J. (1974) Multiple hamartoma syndrome (Cowden disease). *Archives of Dermatology*, **109**, 525–525.

Lloyd, K.M. & Dennis, M. (1963) Cowden's disease: a possible new symptom complex with multiple system involvement. *Annals of Internal Medicine*, **58**, 136–142.

Rosenbluth, M. (1963) Multiple noduli cutanei with gingival manifestations. *Periodontics*, **1**, 81–83.

III.C.5. (*II.A.10.*) *Peutz–Jeghers syndrome* (*intestinal polyposis II*) (AD*)

See II.A.10.

III.D. Disorders Producing Bullae and Vesicles

III.D.1. (III.C.2., III.H.1.) Acrodermatitis enteropathica (AR*)

A syndrome of skin lesions, loss of hair, abnormalities of the nails, and gastrointestinal disturbances. The disorder normally appears in early life with an erythematous then vesiculo-bullous rash, particularly around the eyes and nose and on the hands and feet. The gastrointestinal disturbances manifest themselves in bouts of diarrhoea. In the mouth, the buccal mucosa, and less often the palate and gingiva, are affected by oedema and erosions. There may be numerous small whitish papillomas on the buccal mucosa and borders of the tongue. Over half the cases reported have suffered from secondary candidosis.

McK 20110 *OFG* 354–356 *SHN* 17–20

Gorlin, R.J. (1969) Genetic disorders affecting mucous membranes. *Oral Surgery*, **28**, 512–525.

III.D.2. Dyskeratosis congenita (XL*)

A combination of hyperpigmentation of the skin, dystrophy of the nails, and oral leucoplakia. There may also be continuous lacrimation due to atresia of the lacrimal ducts, thrombocytopenia, anaemia and testicular atrophy. In the mouth, recurrent crops of painless vesicles and bullae appear during childhood. After several attacks the mucosa becomes atrophic, thickened, fissured, and white in patches. The tongue loses its papillae and becomes smooth. Verrucous carcinoma of the oral mucosa has been reported in some cases.

McK 30500 *OFG* 338–340 *SHN* 258–261

Cannell, H. (1971) Dyskeratosis congenita. *British Journal of Oral Surgery*, **9**, 8–20.

Inoue, S., Mekanik, G., Mahallati, M. & Zuelzer, W.W. (1973) Dyskeratosis congenita with pancytopenia. *American Journal of Diseases of Children*, **126**, 389–396.

Milgrom, H., Stoll, H.L. & Crissey, J.T. (1964) Dyskeratosis congenita. *Archives of Dermatology*, **89**, 345–349.

Wald, C. & Diner, H. (1974) Dyskeratosis congenita with associated periodontal disease. *Oral Surgery*, **37**, 736–744.

III.D.3. (IV.D.8., IV.E.2.) Epidermolysis bullosa (H)

A group of several hereditary vesicular disorders involving the skin, and sometimes the oral and other mucosas. Cutaneous vesicles often arise at sites of normal friction and following trauma, but may also appear spontaneously or in response to heat. Rupture of bullae exposes a raw and painful surface, and subsequent healing can produce gross keloidal scarring, contraction, and pigmentation or depigmentation. The nails may be thickened and dystrophic. Oral bullae occur with different frequency in the different forms. In some forms, particularly the recessive dystrophic type, even the manipulations involved in routine dental treatment can cause the eruption of bullae on the lips and oral mucosa. There may also be enamel hypoplasia, and overproduction of poorly calcified cellular cementum. A full classification of these disorders can be found in McKusick's *Mendelian Inheritance in Man* (fifth edition, 1978), Table A-III, page xxxvii.

OFG 201–206, 281, 346–349 *SHN* 281–288

Arwill, T., Olsson, O. & Bergenholtz, A. (1965) Epidermolysis bullosa hereditaria. III. A histologic study of changes in teeth in the polydysplastic, dystrophic and lethal forms. *Oral Surgery*, **19**, 723–744.

Brain, E.B. & Wigglesworth, J.S. (1968) Developing teeth in epidermolysis bullosa hereditaria letalis; a histologic study. *British Dental Journal*, **124**, 255–260.

Gardner, D.G. & Hudson, C.D. (1975) The disturbances in odontogenesis in epidermolysis bullosa hereditaria letalis. *Oral Surgery*, **40**, 483–493.

Giansanti, J.S. (1975) Oral nodular excrescences in epidermolysis bullosa. *Oral Surgery*, **40**, 385–390.

Gormley, J.W. & Schow, C.E. (1976) Epidermolysis bullosa and associated problems in oral surgical treatment. *Journal of Oral Surgery*, **34**, 45–52.

Hitchin, A.D. (1973) The defects of cementum in epidermolysis bullosa dystrophica. *British Dental Journal*, **135**, 437–442.

Morgan, W.C. (1975) Dental anesthetic management in epidermolysis bullosa: a new approach. *Oral Surgery*, **40**, 732–735.

Winstock, D. (1962) Oral aspects of epidermolysis bullosa. *British Journal of Dermatology*, **74**, 431–438.

III.D.4. Familial benign chronic pemphigus (Hailey–Hailey disease) (AD*)

A relatively benign disorder in which there are recurrent vesicular eruptions on the skin, particularly of the neck, groin and axillary regions. Oral involvement has been reported.

McK 16960

Berger, R.S. & Lynch, P.J. (1971) Familial benign chronic pemphigus. Surgical treatment and pathogenesis. *Archives of Dermatology*, **104**, 380–384.

Botvinick, I. (1973) Familial benign pemphigus with oral mucous membrane lesions. *Cutis*, **12**, 371–373.

Izumi, A.K., Shmunes, E. & Wood, M.G. (1971) Familial benign chronic pemphigus. The role of trauma including contact sensitivity. *Archives of Dermatology*, **104**, 177–181.

Sire, D.J. & Johnson, B.L. (1971) Benign familial chronic pemphigus treated with dapsone. *Archives of Dermatology*, **103**, 262–265.

III.D.5. (IV.F.3., IV.H.5.) Porphyria (H)

See IV.F.3.

III.E. Disorders Producing Vascular Lesions

III.E.1. Ataxia telangiectasia (AR*)

A syndrome of progressive cerebellar ataxia, oculocutaneous telangiectasia and sinopulmonary infections. Venous telangiectasia usually appears in the temporal and nasal areas of the conjunctiva in early childhood. The cutaneous telangiectasia appears first on the ears, then on the face, and later extends to the neck and elsewhere. In some cases the telangiectases may not be conspicuous. If the nasal mucosa is involved, there may be recurrent bouts of epistaxis. Telangiectasia of the hard palate has been reported. There is a tendency for affected individuals to develop lymphatic malignancy, including lymphosarcoma of the palate.

McK 20890 *SHN* 37–41

Boder, E. (1975) Ataxia-telangiectasia: some historic, clinical and pathologic observations. *Birth Defects*, **11**(1), 255–270.

Gimeno, A., Liaño, H. & Kreisler, M. (1969) Ataxia-telangiectasia with absence of IgG. *Journal of Neurological Science*, **8**, 545–554.

Reye, C. (1960) Ataxia-telangiectasia. *American Journal of Diseases of Children*, **99**, 238–241.

Smeby, B. (1966) Ataxia-telangiectasia. *Acta Paediatrica Scandinavica*, **55**, 239–243.

III.E.2. (IV.G.1.) Fabry disease (diffuse angiokeratoma) (XL*) (see also Chapter 4)

A disorder in which there is abnormal accumulation of the glycosphingolipid trihexosyl ceramide, particularly in the cardiovascular system and kidneys, due to a deficiency of the enzyme α-galactosidase A. Clusters of macular to papular, punctate, dark red, keratotic angiectases appear in the superficial layers of the skin, usually during childhood. These lesions do not blanch with pressure. Dilation and tortuosity of conjunctival and retinal vessels, and corneal opacities, develop. There is progressive deterioration of renal function, renal failure being the usual cause of death. Periodic episodes of severely painful paraesthesias occur, probably due to lipid changes in the ganglia of the autonomic nervous system. There are usually pinpoint macular purplish spots on the lips and, less frequently, on the buccal mucosa. Heterozygous females may be mildly affected by the disorder, though they rarely show skin lesions. A diagnosis of hemizygosity for Fabry disease can be confirmed by histochemical and biochemical analysis of the dental pulp.

MBID 810–840 *McK* 30150 *OFG* 294–298, 349–351 *SHN* 295–299

Brindley, H.P., Archard, H.O., Alling, C.C., Jurgens, P.E. & Jurgens, E.H. (1975) Angiokeratoma corporis diffusum (Fabry's disease). *Journal of Oral Surgery*, **33**, 199–205.

Desnick, S.J., Witkop, C.J. Jr, Krivit, W., Thies, J.K. & Desnick, R.J. (1972) Fabry's disease: diagnostic confirmation by analysis of dental pulp. *Archives of Oral Biology*, **17**, 1473–1479.

Wise, D., Wallace, H.J. & Jellinek, E.H. (1962) Angiokeratoma corporis diffusum: a clinical study of eight affected families. *Quarterly Journal of Medicine*, **55** (N.S.**31**), 177–206.

Witkop, C.J. Jr (1971) Manifestations of genetic diseases in the human pulp. *Oral Surgery*, **32**, 278–316.

III.E.3. Hereditary haemorrhagic telangiectasia (AD*) (see also Chapter 4)

A condition in which there are multiple capillary venous dilations of the skin and mucous membranes, which may be accompanied by a variety of internal vascular anomalies. The cutaneous and mucosal lesions may take different forms, being pinpoint, or spider like, or nodular. These lesions blanch on pressure. Skin telangiectases usually appear in adolescence or early adult life, particularly on the face, and increase in number and severity with age. Involvement of the nasal mucosa leads to recurrent bouts of epistaxis, and gastric mucosal involvement may result in haematemesis and melena. In the mouth, the tip and dorsum of the tongue and mucosal surfaces of the lips are most frequently affected, though other oral sites may also be involved.

McK 18730 *OFG* 353–354, 440–441 *SHN* 352–355

Caldwell, T.A., Schweber, S.J. & Lucchesi, F.J. (1970) Resection of tongue lesion associated with hereditary telangiectasia (Osler–Weber–Rendu disease): report of a case. *Journal of Oral Surgery*, **28**, 299–301.

Donaldson, D. & Myall, R.W.T. (1973) Hereditary hemorrhagic telangiectasia, Raynaud's disease and the C.R.S.T. syndrome. *Oral Surgery*, **36**, 512–516.

Durocher, R.T., Moeeis, A.L. & Burket, L.W. (1961) Oral manifestations of hereditary hemorrhagic telangiectasia. *Oral Surgery*, **14**, 550–555.

Hashimoto, K. & Pritzker, M.S. (1972) Hereditary hemorrhagic telangiectasia. *Oral Surgery*, **34**, 751–768.

Soudah, H.P. & Tilson, H.B. (1971) Hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease): discussion and report of a case. *Journal of Oral Surgery*, **29**, 225–229.

III.F. Disorders Resulting in Mucosal Fragility

III.F.1. (IV.D.6., IV.H.1., V.A.1.) Ehlers–Danlos syndromes (H)

A series of disorders in which there is hyperelasticity and fragility of the skin, cutaneous and internal haemorrhages, and loose jointedness. The oral mucosa is also unusually fragile and easily bruised. If surgery is required there may be operative difficulties due to abnormal bleeding and because sutures do not hold satisfactorily. There may be severe bleeding following tooth extraction. The teeth may have stunted and deformed roots and contain large pulp stones, resembling the abnormal teeth found in cases of dentine dysplasia type I (see IV.C.1.). The enamel may be hypoplastic. Periodontal disease occurs at an early age in some cases, and, in a few of these, periodontal destruction may be severe. Recurrent dislocation of the temporomandibular joint has also been reported. The classification of Ehlers–Danlos syndromes, with modes

of inheritance, reference numbers in McKusick's catalogue, and associated biochemical defects, is as follows:

Types I, II, III (AD*) *McK* 13000 Biochemical defect unknown
Type IV (AD) *McK* 13005, 22535 Type 3 collagen deficiency
Type V (XL*) *McK* 30520 Lysyl oxidase deficiency?
Type VI (AR*) *McK* 22540 Protocollagen lysyl hydroxylase deficiency
Type VII (AR*) *McK* 22541 Procollagen peptidase deficiency
Type VIII (AD*) *McK* 13008 Biochemical defect unknown

OFG 257, 340–344 *SHN* 262–269

Barabas, G.M. (1969) The Ehlers–Danlos syndrome: abnormalities of the enamel, dentine, cementum and the dental pulp: a histological examination of 13 teeth from 6 patients. *British Dental Journal*, **126**, 509–515.
Barabas, G.M. & Barabas, A.P. (1967) The Ehlers–Danlos syndrome: a report of the oral and haematological findings in nine cases. *British Dental Journal*, **123**, 473–479.
Goodman, R.M. & Allison, M.L. (1969) Chronic temporomandibular joint subluxation in Ehlers–Danlos syndrome. *Journal of Oral Surgery*, **27**, 659–661.
Hughes, C.L. (1970) Odontectomy in treatment of Ehlers–Danlos syndrome: report of a case. *Journal of Oral Surgery*, **28**, 612–614.
Recant, B.S. & Lipman, J.S. (1969) The Ehlers–Danlos syndrome. *Oral Surgery*, **28**, 460–463.
Stewart, R.E., Hollister, D.W. & Rimoin, D.L. (1977) A new variant of Ehlers–Danlos syndrome: an autosomal dominant disorder of fragile skin, abnormal scarring, and generalized periodontitis. *Birth Defects*, **13** (3B), 85–93.
Thexton, A.A. (1965) A case of Ehlers–Danlos syndrome presenting with recurrent dislocation of the temporomandibular joint. *British Journal of Oral Surgery*, **2**, 190–193.

III.G. Metabolic Disorders with Associated Oral Ulceration

III.G.1. Acatalasia (AR*)

A deficiency of the enzyme catalase. In normal individuals, hydrogen peroxide produced by oral microorganisms is broken down by blood and tissue catalase into water and oxygen. In the absence of catalase activity, hydrogen peroxide accumulates in the tissues and is thought to cause local tissue destruction, probably both directly and through breakdown of haemoglobin with a consequently reduced local oxygen tension. The resulting necrosis favours further proliferation of microorganisms and therefore further hydrogen peroxide production. The enzyme deficiency is associated with varying degrees of severity of oral ulceration, but only in about 75 per cent of individuals homozygous for the abnormal, zero-catalase-producing, allele. There are no other signs elsewhere in the body, and the remaining 25 per cent of abnormal homozygotes are completely asymptomatic. There is evidence for different forms of the enzyme deficiency, some abnormal homozygotes showing low levels of catalase rather than zero catalase activity.

MBID 1792–1807 *McK* 20020 *OFG* 371–372, 402, 424

Hamilton, H.B. & Neel, J.V. (1963) Genetic heterogeneity in human acatalasia. *American Journal of Human Genetics*, **15**, 408–419.
Takahara, S. (1954) Progressive oral gangrene due to acatalasemia. *Laryngoscope*, **64**, 685–688.

III.G.2. (IV.I.8.) Cystinosis (H)

A condition in which there is abnormal deposition of cystine crystals in the body tissues, growth retardation, signs resembling those of vitamin D-resistant rickets, and photophobia. There may be associated stomatitis, retarded dental calcification and delayed eruption of the teeth. Three autosomal recessive forms of the disorder have been described, but in none is the basic biochemical defect understood.

MBID 1660–1682 *McK* 21975 (AR*), 21980 (AR*), 21990 (AR*) *OFG* 398–399

Nazif, M. & Osman, M. (1973) Oral manifestations of cystinosis. *Oral Surgery*, **35**, 330–338.

III.H. Disorders Increasing Susceptibility to Candidosis

III.H.1. (III.C.2., III.D.1.) Acrodermatitis enteropathica (AR*)

See III.D.1.

III.H.2. (I.A.11., IV.A.5., IV.D.5.) Ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome (AD*)

See I.A.11.

III.H.3. (IV.D.7.) Endocrine candidosis syndrome (AR*)

A combination of Addison's disease, hypoparathyroidism and superficial candidosis. Candidosis usually appears during the first few months of life, the endocrine abnormalities manifesting themselves either months or years later, though usually before puberty. Hypoparathyroidism nearly always produces tetany, and Addison's disease results in progressive weakness, anorexia, hypotension and pigmentation of the skin and mucous membranes. Death is usually the result of an adrenal crisis. The skin, nail beds, and oral and other mucosae are affected by candidosis. In the mouth, thick creamy white plaques appear, surrounded by reddened hyperaemic areas. The teeth may have chalky and hypoplastic enamel.

McK 24030 *OFG* 344–346 *SHN* 270–275

Castells, S., Fikrig, S., Inamdar, S. & Orti, E. (1971) Familial moniliasis, defective delayed hypersensitivity, and adrenocorticotrophic hormone deficiency. *Journal of Pediatrics*, **79**, 72–79.

Greenberg, M.S., Brightman, V.J., Lynch, M.A. & Ship, I.I. (1969) Idiopathic hypoparathyroidism, chronic candidosis and dental hypoplasia. *Oral Surgery*, **28**, 42–53.

Nally, F.F. (1970) Idiopathic juvenile hypoparathyroidism with superficial moniliasis. *Oral Surgery*, **30**, 356–365.

Perheentupa, J. & Hiekkala, H. (1973) Twenty cases of the syndrome of autoimmune endocrinopathy and candidiasis. *Acta Paediatrica Scandinavica*, **62**, 110–111.

III.H.4. Familial chronic mucocutaneous candidosis, autosomal dominant type (AD)

Early onset, mild mucocutaneous candidosis with increased susceptibility to bacterial infection, follicular hyperkeratosis, universal alopecia, keratoconjunctivitis, diarrhoea in infancy, T- and B-cell abnormalities and possible hypoadrenalism.

McK 11458

Okamoto, G.A., Hall, J.G., Ochs, H., Jackson, C., Rodaway, K. & Chandler, J. (1977) New syndrome of chronic mucocutaneous candidiasis. *Birth Defects*, **13**(3B), 117–125.

III.H.5. Familial chronic mucocutaneous candidosis, autosomal recessive type (AR*)

A disorder distinct from candidosis with endocrinopathy. The condition affects the nails and skin as well as the mucous membranes, and has been found to be associated with iron deficiency in a high proportion of cases.

McK 21205

Higgs, J.M. & Wells, R.S. (1972) Chronic mucocutaneous candidiasis: associated abnormalities of iron metabolism. *British Journal of Dermatology*, **86** (Supplement 8), 88–102.

Wells, R.S. (1970) Chronic oral candidiasis (autosomal recessive inheritance). *Proceedings of the Royal Society of Medicine*, **63**, 890–891.

Wells, R.S., Higgs, J.M., MacDonald, A., Valdimarsson, H. & Holt, P.J.L. (1972) Familial chronic mucocutaneous candidiasis. *Journal of Medical Genetics*, **9**, 302–310.

III.H.6. Myeloperoxidase deficiency (AR*)

Lack of activity of the lysosomal enzyme myeloperoxidase in neutrophils and monocytes, associated with disseminated candidosis.

McK 25460

Klebanoff, S.J. & Pincus, S.H. (1971) Hydrogen peroxide utilization in myeloperoxidase-deficient leukocytes: a possible microbicidal control mechanism. *Journal of Clinical Investigation*, **50**, 2226–2229.

Lehrer, R.I. & Cline, M.J. (1969) Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to Candida infection. *Journal of Clinical Investigation*, **48**, 1478–1488.

Salmon, S.E., Cline, M.J., Schultz, J. & Lehrer, R.I. (1970) Myeloperoxidase deficiency: immunologic study of a genetic leukocyte defect. *New England Journal of Medicine*, **282**, 250–253.

Valdimarsson, H., Moss, P.D., Holt, P.J.L. & Hobbs, J.R. (1972) Treatment of chronic mucocutaneous candidiasis with leukocytes from HL-A compatible siblings. *Lancet*, **i**, 469–472.

SINGLE GENE DISORDERS AFFECTING THE TEETH

IV.A. Disorders Affecting the Number, Size or Shape of Teeth

IV.A.1. Achondroplasia (AD*)

A quantitative reduction in endochondral ossification, leading to short-limbed dwarfism, an enlarged head, depressed nasal bridge, lumbar lordosis, prominent buttocks and protruding abdomen. Tapering upper incisor crowns and other dental anomalies have been reported, but only in two cases.

McK 10080 *OFG* 523–524 *SHV* 12–16

Brook, A.H. & Winter, G.B. (1970) Dental anomalies in association with achondroplasia. *British Dental Journal*, **129**, 519–520.

IV.A.2. (*II.A.2., IV.D.3., IV.J.1.*) *Chondroectodermal dysplasia* (*Ellis–van Creveld syndrome*) (AR*)

See II.A.2.

IV.A.3. (*I.B.2., IV.D.4., IV.E.1., IV.I.4.*) *Cleidocranial dysplasia* (*cleidocranial dysostosis*) (AD*)

A combination of aplasia or hypoplasia of the clavicles, exaggerated transverse diameter of the cranium, delayed ossification of the fontanels, and other skeletal abnormalities. There is usually short stature, a depressed nasal bridge, maxillary hypoplasia, and a highly arched palate that may have a submucous or even a

complete cleft. Tooth eruption is delayed or may fail to occur altogether. Cysts may develop around the unerupted teeth. Supernumerary teeth are frequent. The enamel may be hypoplastic, and the roots regularly lack the normal layer of cellular cementum.

McK 11960 *OFG* 140, 282–284, 566–567 *SHN* 180–184

Bjorn, H. & Grahnén, H. (1966) Cleido-cranial dysostosis. *Odontologisk Revy*, **17**, 67–74.

Hitchin, A.D. & Fairley, J.M. (1974) Dental management in cleidocranial dysostosis. *British Journal of Oral Surgery*, **12**, 46–55.

Kalliala, E. & Taskinen, P.J. (1962) Cleidocranial dysostosis. Report of six typical cases and one atypical case. *Oral Surgery*, **15**, 808–822.

Magnus, W.W. & Sands, W.R. (1974) Cleidocranial dysostosis. Report of a case. *American Journal of Orthodontics*, **65**, 638–643.

Rushton, M.A. (1937) The failure of eruption in cleidocranial dysostosis. *British Dental Journal*, **63**, 641–645.

Rushton, M.A. (1956) An anomaly of cementum in cleidocranial dysostosis. *British Dental Journal*, **100**, 81–83.

Smith, N.H. (1968) A histological study of cementum in a case of cleidocranial dysostosis. *Oral Surgery*, **25**, 470–478.

Winter, G.R. (1943) Dental conditions in cleidocranial dysostosis. *American Journal of Orthodontics*, **29**, 61–89.

IV.A.4. Craniofacial dysostosis (Crouzon syndrome) (AD*)

A syndrome of craniosynostosis, maxillary hypoplasia, ocular hypertelorism, and shallow orbits with consequent ocular proptosis. There is no characteristically shaped calvarium, the nature of the cranial deformity depending on the sutures involved. Raised intracranial pressure with epilepsy and mental deficiency may be observed in some cases. Missing teeth, peg-shaped teeth and macrodontia have been reported.

McK 12350 *OFG* 569, 574–576 *SHN* 220–223

Cohen, M.M. Jr (1975) An etiologic and nosologic overview of craniosynostosis syndromes. *Birth Defects*, **11**(2), 137–189.

Dodge, H.W., Wood, M.W. & Kennedy, R.L.J. (1959) Craniofacial dysostosis: Crouzon's disease. *Pediatrics*, **23**, 98–106.

Gustafson, M.O., Petersen, J.K., Banasik, P.M. & Laskin, D.M. (1971) Surgical correction for craniofacial dysostosis: report of a case. *Journal of Oral Surgery*, **29**, 217–222.

Kelln, E.E., Chaudhry, A.P. & Gorlin, R.J. (1960) Oral manifestations of Crouzon's disease. *Oral Surgery*, **13**, 1245–1248.

IV.A.5. (I.A.11., III.H.2., IV.D.5.) Ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome (AD*)

See I.A.11.

IV.A.6. (III.C.3., IV.D.9., IV.J.9.) Focal dermal hypoplasia (Goltz syndrome) (XL*)

See III.C.3.

IV.A.7. (IV.J.2.) Hallermann–Streiff syndrome (oculomandibulodyscephaly) (AR)

See IV.J.2.

IV.A.8. Hereditary generalized microdontia (AD or XL)

A condition, reported in seven members of one family over three generations, in which all the teeth are smaller than normal. The distribution in the family was consistent with either autosomal dominant or X-linked dominant inheritance.

McK 15680

Steinberg, A.G., Warren, J.F. & Warren, L.M. (1961) Hereditary generalized microdontia. *Journal of Dental Research*, **40**, 58–62.

IV.A.9. Hypodontia and nail dysplasia (tooth and nail syndrome) (AD*)

A combination of fine hair, missing teeth, and hypoplasia or koilonychia of the nails, particularly the toenails. The teeth most frequently absent are the mandibular incisors, second molars and maxillary canines.

McK 18950 *SHN* 736

Giansanti, J.S., Long, S.M. & Rankin, J.L. (1974) The 'tooth and nail' type of autosomal dominant ectodermal dysplasia. *Oral Surgery*, **37**, 576–582.

Hudson, C.D. & Witkop, C.J. (1975) Autosomal dominant hypodontia with nail dysgenesis. *Oral Surgery*, **39**, 409–423.

Redpath, T.H. & Winter, G.S. (1969) Autosomal dominant ectodermal dysplasia with significant dental defects. *British Dental Journal*, **126**, 123–128.

IV.A.10. Hypodontia, taurodontism and sparse scalp hair (AR)

Hypodontia associated with taurodontism among the teeth that do form. The condition has been reported with and without general manifestations of ectodermal dysplasia.

Stenvik, A., Zachrisson, B.U. & Svatun, B. (1972) Taurodontism and concomitant hypodontia in siblings. *Oral Surgery*, **33**, 841–845.

Stoy, P.J. (1960) Taurodontism associated with other dental abnormalities. *Dental Practitioner*, **10**, 202–205.

IV.A.11. Hypohidrotic (anhidrotic) ectodermal dysplasia (H)

A syndrome of hypodontia, hypotrichosis and hypohidrosis. There is also frontal bossing and a depressed nasal bridge. Scalp hair is sparse and often blond, and eyelashes and eyebrows may be reduced almost to the point of complete absence. The skin is soft and thin.

frequently showing obvious signs of dryness. Inability to sweat can result in severe hyperpyrexia after only mild exertion. The nails are sometimes spoon-shaped. Teeth that do develop are usually of a rudimentary shape, often having conical crowns. There are autosomal recessive and X-linked forms of the disorder, but the X-linked form is by far the most frequent. In the X-linked condition, carrier females may show minimal signs, such as mild hypodontia, conical teeth, and a reduced number of sweat glands.

McK 22490 (AR*), 30510 (XL*) *OFG* 588–589
SHV 379–385

Bartlett, R.C., Eversole, L.R. & Adkins, R.S. (1972) Autosomal recessive hypohidrotic ectodermal dysplasia: Dental manifestations. *Oral Surgery*, **33**, 736–742.

Borjian, H. (1960) The effect of early dental treatment of anhidrotic ectodermal dysplasia. *Journal of the American Dental Association*, **61**, 555–564.

Sackett, L.M., Marans, A.E. & Hursey, R.J. (1956) Congenital ectodermal dysplasia of the anhidrotic type. *Oral Surgery*, **9**, 659–665.

Sarnat, B.G., Brodie, A.G. & Kubacki, W.H. (1953) Fourteen year report of facial growth in a case of complete anodontia with ectodermal dysplasia. *American Journal of Diseases of Children*, **86**, 162–169.

Settineri, W.M.F., Salzano, F.M. & De Melo e Freitas, M.J. (1976) X-linked anhidrotic ectodermal dysplasia with some unusual features. *Journal of Medical Genetics*, **13**, 212–216.

IV.A.12. (IV.I.10.) Incontinentia pigmenti (XL*)

A disorder in which there are vesicular, verrucous and pigmented lesions of the skin in infancy. There may also be mental retardation and abnormalities of the eye, including strabismus, optic atrophy and retinal detachment. Dental abnormalities have been reported in both the primary and permanent dentitions. They include missing teeth, peg-shaped teeth, teeth with conical crowns, and delayed eruption. Some form of dental abnormality has been found in nearly all cases described. The pattern of inheritance is consistent with X-linked dominance, with almost complete lethality in affected males. About 95 per cent of reported cases have been females.

McK 30830 *SHV* 393–396

Foster, S.C. & Album, M.M. (1970) Incontinentia pigmenti: Block-Sultzberger, Block-Seimens disease. *Oral Surgery*, **29**, 837–845.

Gorlin, R.J. & Anderson, J.A. (1960) The characteristic dentition of incontinentia pigmenti. *Journal of Pediatrics*, **57**, 78–85.

Russell, D.L. & Finn, S.B. (1967) Incontinentia pigmenti (Bloch–Sulzberger syndrome): a case report with emphasis on dental manifestations. *Journal of Dentistry for Children*, **34**, 494–500.

IV.A.13. (III.B.1., IV.D.13.) Lipoid proteinosis (hyalinosis cutis et mucosae) (AR*)

See III.B.1.

IV.A.14. (I.A.13., II.A.8., II.B.7.) Oral–facial–digital syndrome I (OFD I syndrome) (XL*)

See I.A.13.

IV.A.15. Otodental dysplasia (globodontia) (AD*)

A combination of high-frequency hearing loss and strikingly large, globe-shaped posterior teeth with abnormal cusp patterns.

McK 16675 *OFG* 154–155, 290

Levin, L.S., Jorgenson, R.J. & Cook, R.A. (1975) Otodental dysplasia: a 'new' ectodermal dysplasia. *Clinical Genetics*, **8**, 136–144.

Witkop, C.J. Jr, Gundlach, K.K.H., Streed, W.J. & Sauk, J.J. Jr (1976) Globodontia in the otodental syndrome. *Oral Surgery*, **41**, 472–483.

IV.A.16. Premolar aplasia, hyperhidrosis and canities prematura (PHC syndrome; Böök syndrome) (AD*)

A combination of absence of one or more premolars, hyperhidrosis, and premature greying of the hair, reported in 25 individuals over four generations in a Swedish family.

McK 11230 *OFG* 137

Böök, J.A. (1950) Clinical and genetical studies of hypodontia. I. Premolar aplasia, hyperhidrosis, and canities prematura. A new hereditary syndrome in man. *American Journal of Human Genetics*, **2**, 240–263.

IV.A.17. Pyramidal molar roots, juvenile glaucoma and unusual morphology of upper lip (Ackerman syndrome) (AD)

Pyramidal, taurodont or fused molar roots, associated with juvenile glaucoma, sparse body hair, and a full upper lip lacking a cupid's bow.

SHV 731

Ackerman, J.L., Ackerman, A.L. & Ackerman, A.B. (1973) Taurodont, pyramidal and fused molar roots associated with other anomalies in a kindred. *American Journal of Physical Anthropology*, **38**, 681–694.

IV.A.18. Rieger syndrome (AD*)

A syndrome of hypodontia, dysgenesis of the iris and cornea, and myotonic dystrophy. There may be associated maxillary hypoplasia. The teeth most frequently missing are the maxillary incisors and second premolars. Conical crown form has also been reported.

McK 18050 *SHV* 649–651

Feingold, M., Shiere, F., Fogels, H.R. & Donaldson, D. (1969)

Rieger's syndrome. *Pediatrics*, **44**, 564–569.
Langdon, J.D. (1970) Rieger's syndrome. *Oral Surgery*, **30**, 788–795.
Sedeghi-Najar, A. & Senior, B. (1974) Autosomal dominant transmission of isolated growth hormone deficiency in iris-dental dysplasia (Rieger's syndrome). *Journal of Pediatrics*, **85**, 644–648.
Wilson, J.P. (1955) A case of partial anodontia. *British Dental Journal*, **99**, 199–200.

IV.A.19. Total absence of permanent teeth (AR)

Total failure of development of the permanent teeth, with no obvious associated defects, has been reported in the offspring of a first cousin marriage and in two members of a sibship of seven. These observations are consistent with autosomal recessive inheritance, but require confirmation.

Cramer, M. (1947) Case report of complete anodontia of the permanent teeth. *American Journal of Orthodontics*, **33**, 760–764.
Warr, V.C. (1938) A case of complete absence of permanent dentition. *British Dental Journal*, **64**, 327–328.

IV.B. Primary Disorders of Enamel Formation

IV.B.1. (IV.I.1.) Amelogenesis imperfecta, hypocalcified type (AD*)

This is the most common form of amelogenesis imperfecta. The enamel on newly erupted teeth is usually of normal thickness, though there may be mild hypoplasia. However, the enamel is so soft that it is rapidly lost once exposed to attrition. There may be a delay or failure of eruption. Patients with this condition appear to be unusually prone to develop calculus, particularly on the mandibular teeth. There is some evidence for a recessive form of the disorder.

McK 10450 *OFG* 188–193

Chabora, A.J., Berkman, M.D., Horowitz, S.L. & Nahoum, H.I. (1972) Hereditary hypocalcified amelogenesis imperfecta. Pedigree analysis. *Oral Surgery*, **33**, 922–925.
Darling, A.I. (1956) Some observations on amelogenesis imperfecta and calcification of the dental enamel. *Proceedings of the Royal Society of Medicine*, **49**, 759–765.
Rushton, M.A. (1964) Hereditary enamel defects. *Proceedings of the Royal Society of Medicine*, **57**, 53–58.
Witkop, C.J. Jr & Rao, S. (1971) Inherited defects in tooth structure. *Birth Defects*, **7**(7), 153–184.

IV.B.2. Amelogenesis imperfecta, hypomaturation types (H)

These are disorders in which the enamel is generally of normal thickness, and in which it has an opaque, mottled, brownish-yellow to white appearance. The enamel is softer than normal, though usually not as soft as in cases of hypocalcification amelogenesis imperfecta, and tends to chip from the dentine.

Autosomal recessive pigmented hypomaturation form (AR*). Newly erupted teeth have a milky to shiny brown appearance, but darken further with time due to extrinsic staining. Ground sections of unerupted teeth show a localized band of brown pigment midway between the enamel surface and the enamel–dentine junction. Patients with this condition tend to form large amounts of calculus that is itself often pigmented and that shows an intense red-violet fluorescence.

McK 20470 *OFG* 182–187

Snow capped teeth (AD). A fairly common disorder in which varying proportions of the enamel on the incisal or occlusal surfaces of the teeth have an opaque white appearance. Maxillary teeth tend to be more severely affected than mandibular teeth. The distribution of the defect is not consistent with a timed environmental insult affecting the dentition as a whole. The defect involves surface enamel only, and appears to be due to an abnormality in the final stages of enamel formation within each tooth.

McK 18230 *OFG* 187–188

X-linked hypomaturation amelogenesis imperfecta (XL*). The permanent teeth of affected males are mottled yellow-white, darkening due to extrinsic staining with age. The primary teeth are opaque white with translucent white mottling. In heterozygous females the enamel shows irregular vertical bands of opaque white and normal translucent enamel, and scanning electron microscopy and calcium microanalysis have confirmed that these bands correspond with two distinct types of enamel occurring side by side in the subsurface layers. These findings are consistent with random inactivation of one or other of the X-chromosomes in the somatic cells of heterozygous females. The defect, which appears to be restricted to the enamel rod sheaths, occurs primarily in the outer layers of the enamel.

McK 30110 *OFG* 174–181

McLarty, E.L., Giansanti, J.S. & Hibbard, E.D. (1973) X-linked hypomaturation type of amelogenesis imperfecta exhibiting lyonization in affected females. *Oral Surgery*, **36**, 678–685.
Sauk, J.J., Lyon, H.W. & Witkop, C.J. Jr. (1972) Electron optic microanalysis of two gene products in enamel of females heterozygous for X-linked hypomaturation amelogenesis imperfecta. *American Journal of Human Genetics*, **24**, 267–276.
Winter, G.B. & Brook, A.H. (1975) Enamel hypoplasia and anomalies of the enamel. In *Symposium on Genetics* (Ed.) Poole, A.E. *Dental Clinics of North America*, **19**(1), 3–24.
Witkop, C.J. Jr & Rao, S. (1971) Inherited defects in tooth structure. *Birth Defects*, **7**(7), 153–184.

IV.B.3. (IV.I.2.) Amelogenesis imperfecta, hypoplastic types (H)

These are disorders in which the enamel does not reach normal thickness, either over the whole crown of each tooth, or in localized areas, resulting in pitting or

grooving of the enamel surface. Inherited hypoplastic enamel defects can be divided into three classes:

Autosomal dominant, smooth, rough or pitted forms (AD*). In the smooth form the enamel is thin, hard and glossy, with the morphology of the tooth crowns being little different from that of the enamel–dentine junction. There may be an associated delay or failure of tooth eruption, and pulpal calcifications may be present in both unerupted and erupted teeth. In the rough form the enamel is thin but has a rough granular surface. In the pitted form the enamel approaches normal thickness but has pinpoint to pinhead-sized pits distributed over its surface. The relationship between these different forms is not known.

McK 10453 *OFG* 157–158, 161–167

Autosomal recessive rough amelogenesis imperfecta (enamel agenesis) (AR). In this type of amelogenesis imperfecta there is an almost complete lack of enamel formation. Failure of eruption is common, the unerupted teeth frequenrly undergoing resorption.

OFG 167–169

Hereditary localized enamel hypoplasia (AD). Here the hypoplastic defect manifests itself as a horizontal row of pits, linear depressions, or one large hypoplastic area, with hypocalcification of the adjacent enamel. These defects are most common on the middle thirds of the buccal and labial surfaces of the tooth crowns. The pattern of defects in a given dentition does not conform to what would be expected of an environmental insult at a particular stage of development.

McK 13090 *OFG* 158–161

X-linked hypoplastic amelogenesis imperfecta (XL*). In affected males the enamel is thin, smooth, glossy and yellowish brown. In heterozygous females there is a series of alternating irregular vertical bands of normal and hypoplastic enamel, producing irregular vertical grooving of the enamel surface. The appearance of the enamel in heterozygous females is consistent with random inactivation of one or other of the X-chromosomes.

McK 30120 *OFG* 169–173

Berkman, M.D. & Singer, A. (1971) Demonstration of the Lyon hypothesis in X-linked dominant hypoplastic amelogenesis imperfecta. *Birth Defects*, **7**(7), 204–209.

Burzynski, N.J., Gonzalez, W.E. & Snawder, K.D. (1973) Autosomal dominant smooth hypoplastic amelogenesis imperfecta: report of a case. *Oral Surgery*, **36**, 818–823.

Fischman, S.L. & Fischman, B.C. (1967) Hypoplastic amelogenesis imperfecta: report of a case. *Journal of the American Dental Association*, **75**, 929–931.

Sauk, J.J., Vickers, R.A., Copeland, J.S. & Lyon, H.W. (1972) The surface of genetically determined hypoplastic enamel in human teeth. *Oral Surgery*, **34**, 60–68.

Shokeir, M.H.K. (1971) Hereditary enamel hypoplasia. *Clinical Genetics*, **2**, 387–391.

Witkop, C.J. Jr & Rao, S. (1971) Inherited defects in tooth structure. *Birth Defects*, **7**(7), 153–184.

IV.B.4. Amelogenesis imperfecta with epilepsy and mental deterioration (amelocerebro-hypohidrotic syndrome) (AR or XL)

A combination of almost complete absence of enamel, epilepsy and mental deterioration, leading to death before the age of ten years. The condition has been reported in five male siblings. Hypohidrosis was demonstrated in one case.

McK 22675 *OFG* 200–201 *SHN* 767

Kohlschütter, A., Chappuis, D., Meier, C., Tönz, O., Vassella, F. & Herschkowitz, N. (1974) Familial epilepsy and yellow teeth–a disease of the central nervous system associated with enamel hypoplasia. *Helvetica Paediatrica Acta*, **29**, 283–294.

IV.B.5. Amelogenesis imperfecta with taurodontism, curly hair and sclerotic bones (tricho-dento-osseous syndrome) (AD*)

A combination of hypoplastic and hypocalcified enamel, taurodont teeth, tightly curled hair and cortical osteosclerosis.

McK 19032 *OFG* 208–212 *SHN* 745–746

Crawford, J.L. (1970) Concomitant taurodontism and amelogenesis imperfecta in an American caucasian. *Journal of Dentistry for Children*, **37**, 171–175.

Jorgenson, R.J. & Warson, R.W. (1973) Dental abnormalities in the tricho-dento-osseous syndrome. *Oral Surgery*, **36**, 693–700.

Lichtenstein, J.R., Warson, R.W., Jorgenson, R.J., Dorst, J.P. & McKusick, V.A. (1972) The tricho-dento-osseous (TDO) syndrome. *American Journal of Human Genetics*, **24**, 569–582.

Robinson, G.C., Miller, J.R. & Worth, H.M. (1966) Hereditary enamel hypoplasia: its association with characteristic hair structure. *Pediatrics*, **37**, 498–502.

Winter, G.B., Lee, K.W. & Johnson, N.W. (1969) Hereditary amelogenesis imperfecta. A rare autosomal dominant type. *British Dental Journal*, **127**, 157–164.

IV.B.6. Amelogenesis imperfecta with terminal onycholysis (amelo-onychohypohidrotic syndrome) (AD*)

A syndrome of hypoplastic and hypocalcified enamel, onycholysis with subungual hyperkeratosis, seborrhoeic dermatitis of the scalp, and hypohidrosis with rough, dry skin.

McK 10457 *OFG* 194–196 *SHN* 751

Witkop, C.J. Jr, Brearley, L.J. & Gentry, W.C. (1975) Hypoplastic enamel, onycholysis and hypohidrosis inherited as an autosomal dominant trait: a review of ectodermal dysplasia syndromes. *Oral Surgery*, **39**, 71–86.

IV.C. Primary Disorders of Dentine Formation

IV.C.1. Dentine dysplasia (H)

Two distinct forms of this disorder have been described.

Dentine dysplasia, type I (radicular dentine dysplasia) (AD*). The crowns of permanent and deciduous teeth appear normal, though there may be a slight amber translucency. There is a tendency towards complete obliteration of pulp cavities by abnormal dentine before eruption in the permanent teeth. Failure of normal root development results in short roots or 'rootless' teeth that are sometimes associated with multiple periapical cysts in the absence of caries. Because of poor root formation there is frequently severe mobility and malalignment of the teeth. The earliest formed coronal dentine has normal structure and organization, but the later formed dentine, and the dentine that obliterates the pulp chamber, are grossly abnormal. The dentinal tubules seem to have been repeatedly deflected around abnormal calcified masses during dentine formation, producing a characteristic cascaded appearance. The abnormal dentine is less radio-opaque than normal. These abnormalities of dentine also occur in the brachio-skeleto-genital (BSG) syndrome (see IV.D.2.).

McK 12540 *OFG* 237–239

Dentine dysplasia, type II (coronal dentine dysplasia, pulpal dysplasia) (AD*). Primary teeth have a definite translucent amber appearance whereas the permanent teeth appear normal. Obliteration of pulp chambers tends to occur after eruption, particularly in the deciduous teeth, and is not as complete as in the type I form of the disorder. Root length is normal and there are no multiple periapical cysts. The permanent anterior teeth and premolars frequently show a radicular extension of the pulp cavity, producing a thistle-shaped appearance of the pulp chamber in radiographs. Almost all teeth have multiple accumulations of pulp stones. The first formed dentine has a normal structure, but the later formed dentine is either irregularly tubular or atubular and amorphous. 'Pulpal dysplasia' is probably the same condition.

McK 12542, 17875 *OFG* 239–243

Giansanti, J.S. & Allen, J.D. (1974) Dentin dysplasia, Type II, or dentin dysplasia, coronal type. *Oral Surgery*, **38**, 911–917.

McFarlane, M.W. & Cina, M.T. (1974) Dentinal dysplasia: report of a family. *Journal of Oral Surgery*, **32**, 867–869.

Petersson, A. (1972) A case of dentinal dysplasia and/or calcification of the dental papilla. *Oral Surgery*, **33**, 1014–1017.

Rao, S.R., Witkop, C.J. Jr & Yamane, G.M. (1970) Pulpal dysplasia. *Oral Surgery*, **30**, 682–689.

Sauk, J.J., Lyon, H.W., Trowbridge, H.O. & Witkop, C.J. Jr (1972) An electron optic analysis and explanation for the etiology of dentinal dysplasia. *Oral Surgery*, **33**, 763–771.

Shields, E.D., Bixler, D. & El-Kafrawy, A.M. (1973) A proposed classification for heritable human dentine defect with a description of a new entity. *Archives of Oral Biology*, **18**, 543–553.

Wesley, R.K., Wysocki, G.P., Mintz, S.M. & Jackson, J. (1976) Dentin dysplasia Type I. *Oral Surgery*, **41**, 516–523.

Witkop, C.J. Jr & Rao, S. (1971) Inherited defects in tooth structure. *Birth Defects*, **7**(7), 153–184.

IV.C.2. (IV.D.17.) Dentinogenesis imperfecta (H)

Three forms of this disorder have been described. The first is included in this section for the sake of completeness.

Dentinogenesis imperfecta, type I (the dental manifestations of osteogenesis imperfecta) (H). Dentine defects occurring as part of the generalized inherited skeletal disease, osteogenesis imperfecta. The major non-dental manifestations of this disorder are multiple recurrent bone fractures, hyperextensible joints, blue sclerae and progressive deafness. The most common form of the disorder is autosomal dominant with a wide range of expressivity and incomplete penetrance, but there is also a much more rare and severe congenital autosomal recessive type. The disorder is thought to be due to defective maturation of collagen. Dentine defects have been described in about 50 per cent of cases, the deciduous teeth always being more severely affected than the permanent teeth, but no systematic survey of subclinical dentine involvement in the disorder has been carried out. Affected teeth may have a translucent amber appearance and may show a tendency for the enamel to fracture off the underlying dentine. Radiographs may show teeth with short constricted roots and accelerated obliteration of the pulp chambers. The earliest formed dentine is usually normal, but there is a progressive increase in interglobular areas and disorganization of tubule size and direction in the later formed layers.

McK 16620 (AD*), 16622 (AD*), 25940 (AR*), 25941 (AR*) *OFG* 230–232 *SHN* 584–589

Dentinogenesis imperfecta, type II (hereditary opalescent dentine) (AD*). Dentine defects similar to those found in osteogenesis imperfecta (dentinogenesis imperfecta, type I), but without accompanying general manifestations. In contrast to the type I disorder, penetrance appears to be complete, there is a high correlation between affected members of the same family for severity of effect, and deciduous and permanent teeth are equally affected.

McK 12549 *OFG* 232–236

Dentinogenesis imperfecta, type III (brandywine type) (AD). Similar dentine defects to those found in types I and II of the disorder, but with a wider range of severity. An almost unique feature is the occasional occurrence of 'shell teeth' (8/252 cases in the brandywine isolate), in which dentinogenesis ceases after the formation of the initial mantle layer, leaving an abnormally large pulp chamber. No examples of shell teeth have been observed in the dentitions of the large number of individuals shown to be affected by types I and II of the disorder.

However, a condition resembling shell teeth has been reported in an offspring of two individuals affected by dentinogenesis imperfecta type II. Furthermore, most of the cases of shell teeth have come from the brandywine isolate itself, a population showing a high level of inbreeding and an unusually high incidence of dentinogenesis imperfecta. It is therefore possible that shell teeth are the result of homozygosity for dentinogenesis type II.

McK 12550 *OFG* 236–237

Bergman, G., Engfeldt, B. & Sunvall-Hagland, I. (1956) Studies on mineralised dental tissues. VIII. Histologic and microradiographic investigation of hereditary opalescent dentine. *Acta Odontologica Scandinavica*, **14**, 103–117.

Bixler, D., Conneally, P.M. & Christen, A.G. (1969) Dentinogenesis imperfecta: genetic variations in a six generation family. *Journal of Dental Research*, **48**, 1196–1199.

Eastoe, J.E., Martens, P. & Thomas, N.R. (1973) The amino acid composition of human hard tissue collagens in osteogenesis imperfecta and dentinogenesis imperfecta. *Calcified Tissue Research*, **12**, 91–100.

Godfrey, J.L. (1973) A histological study of dentin formation in osteogenesis imperfecta congenita. *Journal of Oral Pathology*, **2**, 95–111.

Gray, P.H.K. (1970) A case of osteogenesis imperfecta associated with dentinogenesis imperfecta dating from antiquity. *Clinical Radiology*, **21**, 106–108.

Heys, F.M., Blattner, R.J. & Robinson, H.B.G. (1960) Osteogenesis imperfecta and odontogenesis imperfecta: clinical and genetic aspects in eighteen families. *Journal of Pediatrics*, **56**, 234–245.

Mars, M., Farrant, S. & Roberts, G.J. (1976) Dentinogenesis imperfecta–report of a five generation family. *British Dental Journal*, **140**, 206–209.

Miller, W.A., Winkler, S., Rosenberg, J.J., Mastracola, R., Fischman, S.L. & Wolfe, R.J. (1973) Dentinogenesis imperfecta traceable through five generations of a part American Indian family. *Oral Surgery*, **35**, 180–186.

Rushton, M.A. (1954) A new form of dentinal dysplasia: shell teeth. *Oral Surgery*, **7**, 543–549.

Sauk, J.J., Witkop, C.J., Brown, D.M. & Kendall, W.C. (1976) Glycosaminoglycans of EDTA soluble and insoluble dentin in dentinogenesis imperfecta Type I. *Oral Surgery*, **41**, 753–757.

Shields, E.D., Bixler, D. & El-Kafrawy, A.M. (1973) A proposed classification for heritable human dentin defects with a description of a new entity. *Archives of Oral Biology*, **18**, 543–553.

Shokeir, M.H.K. (1972) Dentinogenesis imperfecta: severe expression in a probable homozygote. *Clinical Genetics*, **3**, 442–447.

IV.C.3. Fibrous dysplasia of dentine (AD)

A disorder restricted to dentine, apparently due to abnormal collagen formation. The teeth appear outwardly normal, but there are small radiolucent foci within the dentine, associated with disorganization of the dentine matrix and absence of normal regular tubular structure.

OFG 248

IV.D. Generalized Disorders in which Enamel and/or Dentine Formation may be Abnormal

IV.D.1. Aarskog syndrome (XL*)

A combination of short stature, genital anomalies and unusual facies, including broad forehead, ocular hypertelorism, ptosis, a short stubby nose, and poorly formed external ears. Heterozygous females may be mildly affected. Enamel hypoplasia and hypocalcification have been reported.

McK 30540 *SHN* 1–3

Melnick, M. & Shields, E.D. (1976) Aarskog syndrome: new oral-facial findings. *Clinical Genetics*, **9**, 20–24.

IV.D.2. Brachio-skeleto-genital (BSG) syndrome (AR or XL)

A syndrome of severe maxillary hypoplasia, mental retardation, vertebral anomalies, hypospadias, and dentine abnormalities typical of dentine dysplasia type I (see IV.C.1.).

McK 21138 *OFG* 254 *SHN* 743–744

Elsahy, N.I. & Waters, W.R. (1971) The brachio-skeleto-genital syndrome. *Plastic and Reconstructive Surgery*, **48**, 542–550.

IV.D.3. (II.A.2., IV.A.2., IV.J.1.) Chondro-ectodermal dysplasia (Ellis–van Creveld syndrome) (AR*)

See II.A.2.

IV.D.4. (I.B.2., IV.A.3., IV.E.1., IV.I.4.) Cleido-cranial dysplasia (cleidocranial dysostosis) (AD*)

See IV.A.3.

IV.D.5. (I.A.11., III.H.2., IV.A.5.) Ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome (AD*)

See I.A.11.

IV.D.6. (III.F.1., IV.H.1., V.A.1.) Ehlers–Danlos syndromes (H)

See III.F.1.

IV.D.7. (III.H.3.) Endocrine candidosis syndrome (AR*)

See III.H.3.

IV.D.8.* (*III.D.3.*, *IV.E.2.*) *Epidermolysis bullosa (H)

See III.D.3.

***IV.D.9.* (*III.C.3.*, *IV.A.6.*, *IV.I.9.*) *Focal dermal hypoplasia* (*Goltz syndrome*)** (XL*)

See III.C.3.

***IV.D.10. Hypophosphataemia* (*vitamin D-resistant rickets*)** (H)

A disorder characterized by impaired renal reabsorption of inorganic phosphate, resulting in low serum phosphate concentrations and, in some cases, rickets or osteomalacia that do not respond to the normal therapeutic doses of vitamin D. The dentine is poorly calcified and may contain deficient tracts extending from the tips of elongated pulp horns to the dentine–enamel junction. The enamel above these tracts may be hypoplastic or cracked, providing access to the pulp chamber for oral microorganisms. As a consequence, there may be multiple periapical abscesses associated with apparently normal and healthy looking teeth. The classical disorder is X-linked, with heterozygous females usually moderately affected, but there also appears to be a related autosomal dominant condition.

MBID 1537–1562 *McK* 14635 (AD*), 30780 (XL*) *OFG* 249–253, 402

Archard, H.O. & Witkop, C.J. Jr (1966) Hereditary hypophosphatemia (vitamin D resistant rickets) presenting primary dental manifestations. *Oral Surgery*, **22**, 184–193.

Gigliotti, R., Harrison, H., Reveley, R.A. & Drabkowski, A.J. (1971) Familial vitamin D-refractory rickets. *Journal of the American Dental Association*, **82**, 383–387.

Marks, S.C., Lindahl, R.J. & Bowden, J.W. (1965) Dental and cephalometric findings in vitamin D resistant rickets. *Journal of Dentistry for Children*, **32**, 259–265.

Pliskin, M.E., Brown, A.M., Baden, E.E. & Kimball, H.G. (1975) Vitamin D resistant rickets of a young adult patient–a review and case report. *Journal of Oral Medicine*, **30**, 77–80.

Sauk, J.J. & Witkop, C.J. Jr (1973) Electron optic analysis of human dentin in hypophosphatemic vitamin D-resistant rickets (report of a kindred with consanguinity). *Journal of Oral Pathology*, **2**, 203–214.

Soni, N.N. & Marks, S.C. (1967) Microradiographic and polarised light study of dental tissues in vitamin D-resistant rickets. *Oral Surgery*, **23**, 755–762.

Tracy, W.E., Steen, J.C., Steiner, J.E. & Buist, N.R. (1971) Analysis of dentine pathogenesis in vitamin D resistant rickets. *Oral Surgery*, **32**, 38–44.

IV.D.11.* (*IV.E.4.*, *IV.H.2.*) *Hypophosphatasia (H)

A disorder in which there is low serum alkaline phosphatase activity, inadequate mineralization of the bone matrix, urinary excretion of phosphoethanolamine, and abnormalities of the dentine and cementum. Based on age of onset and severity, the disorder has been divided into three types: an infantile type with severe skeletal abnormalities at birth and a high perinatal mortality; a less severe juvenile type, appearing after six months of age; and a mild adult type. Dental abnormalities have been described in the juvenile type only. There is an unusually wide predentine zone, large dentinal tubules and many areas of interglobular dentine. In addition, there may be almost a total lack of cementum and normally attached periodontal fibres, leading to poor support and premature loss of the teeth, particularly in the deciduous dentition. The most common mode of inheritance is autosomal recessive, but autosomal dominant transmission has also been described.

MBID 1340–1349 *McK* 14630 (AD*), 24150 (AR*) *OFG* 275–278, 402–403

Baer, P.N., Brown, N.C. & Hamner, J.E. (1964) Hypophosphatasia. A report of two cases with dental findings. *Periodontics*, **2**, 209–215.

Beumer, J., Trowbridge, H.O., Silverman, S. & Eisenberg, E. (1973) Childhood hypophosphatasia and the premature loss of teeth. A clinical and laboratory study of seven cases. *Oral Surgery*, **35**, 631–640.

Bruckner, R.J., Rickles, N.Y. & Porter, D.R. (1962) Hypophosphatasia with premature shedding of teeth and aplasia of cementum. *Oral Surgery*, **15**, 1351–1369.

Donoff, R.B. & Guralnick, W.C. (1970) Premature deciduous tooth loss in hypophosphatasia. *Journal of Oral Surgery*, **28**, 501–505.

Pimstone, B., Eisenberg, E. & Silverman, S. (1966) Hypophosphatasia: genetic and dental studies. *Annals of Internal Medicine*, **65**, 722–729.

Poland, C., Eversole, L.R., Bixler, D. & Christian, J.C. (1972) Histochemical observations of hypophosphatasia. *Journal of Dental Research*, **51**, 333–338.

IV.D.12. Lacrimo-auriculo-dento-digital* (*LADD*) *syndrome (AD*)

A combination of aplasia or hypoplasia of the lacrimal puncta with obstruction of the nasolacrimal duct, cup shaped external ears and hearing deficit, mild enamel dysplasia, and a variety of digital anomalies.

McK 14973

Hollister, D.W., Klein, S.H., DeJager, H.J., Lachman, R.S. & Rimoin, D.L. (1973) The lacrimo-auriculo-dento-digital syndrome. *Journal of Pediatrics*, **83**, 438–444.

***IV.D.13.* (*III.B.1.*, *IV.A.13.*) *Lipoid proteinosis* (*hyalinosis cutis et mucosae*)** (AR*)

See III.B.1.

***IV.D.14.* (*II.C.2.*) *Mucolipidosis II* (*I-cell disease*)** (AR*)

See II.C.2.

IV.D.15. Mucopolysaccharidosis IV (*Morquio disease*) (AR*)

A syndrome of dwarfism, progressive spinal deformity, broad nose, thick lips and short neck, the head appearing to rest directly on the shoulders. There is progressive hearing loss, corneal clouding, and marked enamel hypoplasia in both deciduous and permanent dentitions. The enamel is hard and of normal radiopacity, but is uniformly very thin, with occasional pitting, particularly on the buccal aspect of the tooth crowns. The teeth appear grey and small, but have an exaggerated morphology reminiscent of the dentine–enamel junction. The disorder is due to deficiency of the enzyme hexosamine 6-sulphatase.

MBID 1282–1307 *McK* 25300 *OFG* 207–208, 614–615 *SHN* 487–490

Garn, S.M. & Hurme, V.O. (1952) Dental defects in three siblings afflicted with Morquio's disease. *British Dental Journal*, **93**, 210–212.

Levin, L.S., Jorgenson, R.J. & Salinas, C.F. (1975) Oral findings in the Morquio syndrome (mucopolysaccharidosis IV). *Oral Surgery*, **39**, 390–395.

Sela, M., Eidelman, E. & Yatziv, S. (1975) Oral manifestations of Morquio's syndrome. *Oral Surgery*, **39**, 583–589.

IV.D.16. Oculo-dento-osseous dysplasia (*oculo-dento-digital syndrome*) (AD*)

A combination of microphthalmia, iris anomalies, thin nose without alar flare, finger and toe anomalies including syndactyly, and gross enamel hypoplasia in both deciduous and permanent dentitions. The enamel is hard but yellow, and has a rough and severely pitted surface.

McK 16420 *OFG* 196–198, 618–619 *SHN* 553–556

Eidelman, E., Chosack, A. & Wagner, M.L. (1967) Orodigitofacial dysostosis and oculodentodigital dysplasia. *Oral Surgery*, **23**, 311–319.

Gorlin, R.J., Meskin, L.H. & St. Geme, J.W. (1963) Oculodentodigital dysplasia. *Journal of Pediatrics*, **63**, 69–75.

Rajic, D.S. & de Veber, L.L. (1966) Hereditary oculodentoosseous dysplasia. *Annals of Radiology*, **9**, 224–231.

Zach, G.A. (1975) Oculodentoosseous dysplasia syndrome. *Oral Surgery*, **40**, 122–125.

IV.D.17. (*IV.C.2.*) *Osteogenesis imperfecta* (H)

See IV.C.2. (dentinogenesis imperfecta, type I).

IV.D.18. (*IV.I.16.*) *Pseudohypoparathyroidism* (*PHP; Albright hereditary osteodystrophy*) (XL or AD)

A hypocalcaemic syndrome resembling hypoparathyroidism but unresponsive to administered parathormone. There is frequently short stature, a rounded face, depressed nasal bridge, shortening of one or more fingers or toes, and sometimes mental retardation. Severe hypocalcaemia leads to muscle cramps and tetanic convulsions. Thin and pitted enamel has been found in one third to one half of the cases reported. Since the age of onset of the disease is variable, teeth that develop early may escape being affected. However, once the disorder is sufficiently established to produce enamel hypoplasia, all teeth formed subsequently appear to show the enamel defect. In addition, affected teeth may have short roots, and show delayed apical closure and retarded eruption. In the dentine of affected teeth interglobular areas are common. The pattern of inheritance is reasonably consistent with X-linked dominance, though females are, on average, more severely affected than males. It is possible that the disorder is in fact a sex-influenced autosomal dominant.

MBID 1350–1365 *McK* 30080 *OFG* 212–218 *SHN* 626–629

Croft, L.K., Witkop, C.J. Jr & Glas, J.-E. (1965) Pseudohypoparathyroidism. *Oral Surgery*, **20**, 758–770.

Ritchie, G.M. (1965) Dental manifestations of pseudohypoparathyroidism. *Archives of Disease in Childhood*, **40**, 565–572.

Witkop, C.J. Jr (1966) Inborn errors of metabolism with particular reference to pseudohypoparathyroidism. *Journal of Dental Research*, **45**, 568–574.

Witkop, C.J. Jr & Rao, S. (1971) Inherited defects in tooth structure. *Birth Defects*, **7**(7), 153–184.

IV.D.19. (*I.C.10., I.D.12., III.B.5.*) *Tuberous sclerosis* (AD*)

See III.B.5.

IV.D.20. Vitamin D-dependent rickets (AR*)

A disorder characterized by hypocalcaemia and hypophosphataemia, developing within the first year of life and responsive to massive doses of vitamin D. If the condition is left untreated, the teeth that calcify postnatally show gross enamel hypoplasia, large pulp chambers with high pulp horns, and delayed apical closure.

McK 26470 *OFG* 218–219

Arnaud, C., Maijer, R., Reade, T., Scriver, C.R. & Whelan, D.T. (1970) Vitamin D dependency: an inherited postnatal syndrome with secondary hyperparathyroidism. *Pediatrics*, **46**, 871–880.

IV.E. Disorders in which Cementum Formation is Abnormal

IV.E.1. (*I.B.2., IV.A.3., IV.D.4., IV.I.4.*) *Cleidocranial dysplasia* (*cleidocranial dysostosis*) (AD*)

See IV.A.3.

IV.E.2.* (*III.D.3.*, *IV.D.3.*) *Epidermolysis bullosa (H)

See III.D.3.

***IV.E.3.* (*I.C.1.*) *Gardner syndrome* (*intestinal polyposis III*)** (AD*)

See I.C.1.

IV.E.4.* (*IV.D.11.*, *IV.H.2.*) *Hypophosphatasia (H)

See IV.D.11.

IV.E.5.* (*IV.I.13.*) *Multiple non-erupting teeth, maxillo-zygomatic hypoplasia and other congenital defects (AR)

See IV.I.13.

***IV.E.6.* (*I.C.7.*) *Paget disease of bone* (*osteitis deformans*)** (AD)

See I.C.7.

IV.F. Metabolic Disorders Resulting in Tooth Pigmentation

IV.F.1. Alcaptonuria (AR*)

A disorder in which homogentisic acid, an intermediary product in the metabolism of phenylalanine and tyrosine, cannot be further metabolized, due to a deficiency of the enzyme homogentisic acid oxidase. Homogentisic acid consequently appears in the urine, which usually darkens slowly on exposure to air. There is associated arthritis, and a generalized greyish to blue-black pigmentation of connective tissues. Intrinsic pigmentation of the teeth has also been reported.

MBID 268–282 *McK* 20350

Vogel, R.I. (1975) Intrinsic and extrinsic discoloration of the dentition (a literature review). *Journal of Oral Medicine*, **30**, 99–104.

***IV.F.2.* (*IV.G.5.*) *Oxalosis* (*hyperoxaluria*)** (H)

A disorder in which oxalate deposits accumulate in the kidney, causing renal damage, renal failure, uraemia and early death. Oxalate deposits are also found in many other organs and tissues throughout the body. There are two forms of the disorder, both autosomal recessive: oxalosis I (glycolic aciduria) and oxalosis II (glyceric aciduria). A slate-grey intrinsic stain of the teeth has been reported. In addition, oxalate crystals have been demonstrated within odontoblasts and dentinal tubules, and within the dental pulp. Oxalosis I is due to a deficiency of the enzyme α-ketoglutarate glyoxylate carboligase, and oxalosis II to a deficiency of the enzyme D-glyceric dehydrogenase.

MBID 182–204 *McK* 25990 (AR*), 26000 (AR*)

Glass, R.T. (1973) Oral manifestations in primary hyperoxaluria and oxalosis. Report of a case. *Oral Surgery*, **35**, 502–509.

IV.F.3.* (*III.D.5.*, *IV.H.5.*) *Porphyria (H)

A group of disorders of porphyrin metabolism. In the congenital erythropoietic form, which is autosomal recessive, the deciduous and permanent teeth show an irregular reddish brown or pinkish discoloration and red fluorescence under ultraviolet light, due to the incorporation of porphyrin during development. The urine is reddish, there is frequently hirsutism, and there may be vesicular or bullous eruptions of the skin, especially after exposure to sunlight, followed by poor healing and severe scarring. There may also be periodontal involvement. Painful bullae of the oral mucosa have been reported following dental treatment in the hepatic form, which appears to be autosomal dominant. A full classification of the porphyrias, including known biochemical defects, can be found in McKusick's *Mendelian Inheritance in Man* (fifth edition, 1978), Table A-VIII, page lii.

MBID 1166–1220 *OFG* 403–404, 430–434

Gilhuus-Moe, O. & Koppang, H.S. (1972) Oral manifestations of porphyria. *Oral Surgery*, **33**, 926–933.

Kench, J.E., Langley, F.A. & Wilkinson, J.F. (1953) Biochemical and pathological studies of congenital porphyria. *Quarterly Journal of Medicine*, N.S. **22**, 285–294.

Rayne, J. (1967) Porphyria erythropoietica. *British Journal of Oral Surgery*, **5**, 68–74.

Waldenstrom, J. & Haeger-Aronsen, B. (1963) Different patterns of human porphyria. *British Medical Journal*, **ii**, 272–276.

IV.G. Disorders with Known Manifestations in the Dental Pulp (Excluding Abnormal Hard Tissue Formation)

***IV.G.1.* (*III.E.2.*) *Fabry disease* (*diffuse angiokeratoma*)** (XL*)

See III.E.2.

IV.G.2. Metachromatic leucodystrophy (H)

A group of autosomal recessive disorders of myelin formation, due to deficiency of the enzyme arylsulphatase A,

the most common of which is the late infantile form. Less common are the juvenile and adult forms, and the combination of metachromatic leucodystrophy with amaurotic idiocy. The late infantile form usually appears towards the end of the second year of life with minor sensory and motor disturbances. There is generally rapid progression of the disorder, with eventual blindness, deafness and widespread paralysis leading to death during childhood. Accumulations of sulphatides, in the form of brown metachromatic granules within the cytoplasm of Schwann cells, have been demonstrated in the peripheral nerves of the dental pulp. These accumulations are diagnostic of the disorder.

MBID 770–809 *McK* 24980 (AR), 25000 (AR), 25010 (AR*), 25020 (AR) *OFG* 292–293

Gardner, D.G. (1967) Pulpectomy as a diagnostic procedure in metachromatic leucodystrophy. *Oral Surgery*, **23**, 379–384.

Gardner, D.G. & Zeman, W. (1965) Biopsy of the dental pulp in the diagnosis of metachromatic leucodystrophy. *Developmental Medicine and Child Neurology*, **7**, 620–627.

IV.G.3. (*I.D.8., II.A.6., II.B.5., II.C.3., IV.I.12.*) *Mucopolysaccharidosis I–H* (*Hurler syndrome*) (AR*)

See I.D.8.

IV.G.4. Niemann–Pick disease (*sphingomyelin lipidosis*) (AR*)

A disorder of lipid metabolism with primarily neurological manifestations, due to deficiency of the enzyme sphingomyelinase. There is widespread intracellular accumulation of lipid, mainly sphingomyelin. The main clinical features are hepatosplenomegaly, retarded physical and mental growth, and severe neurological disturbances. Symptoms generally appear in the first few months of life, and death usually occurs before three years of age. A typical histological feature is the foam cell, usually an enlarged reticuloendothelial cell whose cytoplasm contains numerous droplet-like inclusions of lipid. The foam cell has been demonstrated in the dental pulp of a patient with Niemann–Pick disease.

MBID 718–730 *McK* 25720 *OFG* 294–295

Stewart, R.E. (1970) Dental pulp biopsy in the diagnosis of neurological disorders in childhood. *Journal of Hospital Dental Practice*, **4**, 13–17.

IV.G.5. (*IV.F.2.*) *Oxalosis* (*hyperoxaluria*) (H)

See IV.F.2.

IV.G.6. (*I.D.6.*) *Sickle cell anaemia* (AD*)

See I.D.6.

IV.H. Disorders Affecting the Periodontium

IV.H.1. (*III.F.1., IV.D.6., V.A.1.*) *Ehlers–Danlos syndromes* (H)

See III.F.1.

IV.H.2. (*IV.D.11., IV.E.4.*) *Hypophosphatasia* (H)

See IV.D.11.

IV.H.3. Palmoplantar hyperkeratosis and periodontoclasia (*Papillon–Lefèvre syndrome*) (AR*)

A combination of hyperkeratosis of the palms and soles, with periodontal destruction in both the deciduous and permanent dentitions. The first signs of periodontal and skin involvement generally appear soon after the eruption of the last deciduous molar. Gingival swelling and severe halitosis accompany periodontal destruction, the teeth usually being involved in the order in which they erupt. Most of the deciduous teeth are usually lost before the age of four years. After tooth loss, the gingival inflammation subsides until the permanent teeth erupt, when the process of gingival swelling, periodontal breakdown and tooth loss repeats itself. All the permanent teeth except the third molars are usually lost before 16 years of age.

McK 24500 *OFG* 279–281 *SHV* 373–376

Carvel, R.I. (1969) Palmar-plantar hyperkeratosis and premature periodontal destruction. *Journal of Oral Medicine*, **24**, 73–82.

Galanter, D.R. & Bradford, S. (1969) Hyperkeratosis palmoplantaris and periodontosis: the Papillon-Lefèvre syndrome. *Journal of Periodontology*, **40**, 40–47.

Giansanti, J.S., Hrabak, R.P. & Waldron, C.A. (1973) Palmar-plantar hyperkeratosis and concomitant periodontal destruction (Papillon-Lefèvre syndrome). *Oral Surgery*, **36**, 40–48.

Gorlin, R.J., Sedano, H. & Anderson, V.E. (1964) The syndrome of palmar-plantar hyperkeratosis and premature periodontal destruction of the teeth. *Journal of Pediatrics*, **65**, 895–908.

Smith, P. & Rosenzweig, K.A. (1967) Seven cases of Papillon-Lefèvre syndrome. *Periodontics*, **5**, 42–46.

Wilson, F.M. (1969) Papillon-Lefèvre syndrome. Report of a case. *Oral Surgery*, **28**, 488–492.

IV.H.4. Periodontosis (*familial juvenile periodontosis; Gottlieb syndrome*) (AR or XL)

A condition in which primary periodontal degeneration and alveolar bone loss occur in an otherwise healthy adolescent, without accompanying periodontal inflammation. However, once periodontal pocket formation has occurred, secondary inflammation follows. Both males and females are affected, with a pre-

ponderance of affected females.

McK 26095, 31175 *OFG* 273–274

Benjamin, S.D. & Baer, P.N. (1967) Familial patterns of advanced bone loss in adolescence (periodontosis). *Periodontics*, **5**, 82–88.
Butler, J.H. (1969) A familial pattern of juvenile periodontosis (periodontosis). *Journal of Periodontology*, **40**, 115–118.
Jorgenson, R.J., Levin, L.S., Hutcherson, S.T. & Salinas, C.F. (1975) Periodontosis in sibs. *Oral Surgery*, **39**, 396–402.
Melnick, M., Shields, E.D. & Bixler, D. (1976) Periodontosis: a phenotypic and genetic analysis. *Oral Surgery*, **41**, 32–43.

IV.H.5.* (*III.D.5.*, *IV.F.3.*) *Porphyria (H)

See IV.F.3.

IV.H.6. Universal permanent alopecia, psychomotor epilepsy, pyorrhoea and mental subnormality (AD*)

A syndrome of congenital universal permanent alopecia, frequently associated with psychomotor epilepsy and mental subnormality. Advanced periodontal disease has been observed in all cases.

McK 10413

Shokeir, M.H.K. (1977) Universal permanent alopecia, psychomotor epilepsy, pyorrhea and mental subnormality. *Clinical Genetics*, **11**, 13–17.

IV.I. Disorders Affecting Tooth Eruption

IV.I.1.* (*IV.B.1.*) *Amelogenesis imperfecta, hypocalcified type (AD*)

See IV.B.1.

IV.I.2.* (*IV.B.3.*) *Amelogenesis imperfecta, hypoplastic types (H)

See IV.B.3.: autosomal dominant, smooth, rough or pitted forms; autosomal recessive rough amelogenesis imperfecta.

***IV.I.3.* (*I.B.1.*) *Apert syndrome* (*acrocephalosyndactyly, type I*)** (AD*)

See I.B.1.

***IV.I.4.* (*I.B.2.*, *IV.A.3.*, *IV.D.4.*, *IV.E.1.*) *Cleidocranial dysplasia* (*cleidocranial dysostosis*)** (AD*)

See IV.A.3.

IV.I.5. Complete failure of eruption of permanent teeth (AD)

A condition in which there is complete failure of eruption of permanent teeth, associated with delayed eruption and persistence of the deciduous dentition. Non-eruption of the permanent teeth may be complicated by the development of multiple dentigerous cysts.

McK 12535

Shokeir, M.H.K. (1974) Complete failure of eruption of all permanent teeth: an autosomal dominant disorder. *Clinical Genetics*, **5**, 322–326.

***IV.I.6. Cryptodontic brachymetacarpalia* (*brachydactyly, type E*)** (AD*)

Type E brachydactyly is a combination of brachydactyly, due mainly to short metacarpals and metatarsals, a round face and moderately short stature. There is wide variability in the number of digits affected. Multiple impacted teeth and delayed eruption have been reported in cases that have shown some of the features of type E brachydactyly, and the whole syndrome has been given the name cryptodontic brachymetacarpalia. Affected individuals appear similar to those with pseudohypoparathyroidism (see IV.D.18.).

McK 11330 *OFG* 284

Arvystas, M.G. (1976) Familial generalized delayed eruption of the dentition with short stature. *Oral Surgery*, **41**, 235–243.
Gorlin, R.J., Sedano, H.O. & Vickers, R.A. (1971) Cryptodontic brachymetacarpalia. *Birth Defects*, **7**(7), 200–203.

IV.I.7.* (*III.A.2.*) *Cutis laxa (H)

See III.A.2.

IV.I.8.* (*III.G.2.*) *Cystinosis (H)

See III.G.2.

***IV.I.9.* (*III.C.3.*, *IV.A.6.*, *IV.D.9.*) *Focal dermal hypoplasia* (*Goltz syndrome*)** (XL*)

See III.C.3.

IV.I.10.* (*IV.A.12.*) *Incontinentia pigmenti (XL*)

See IV.A.12.

IV.I.11. Molar 1 reinclusion (AD*)

A condition consisting of recession or reinclusion of the first molars into the alveolar bone following complete

eruption. The condition has been observed in several families.

McK 15795

Bosker, H., Ten Kate, L.P. & Nijenhuis, L.E. (1978) Familial reinclusion of permanent molars. *Clinical Genetics*, **13**, 314–320.

***IV.I.12.* (*I.D.8., II.A.6., II.B.5., II.C.3., IV.G.3.*) *Mucopolysaccharidosis I–H* (*Hurler syndrome*)** (AR*)

See I.D.8.

IV.I.13.* (*IV.E.5.*) *Multiple non-erupting teeth, maxillo-zygomatic hypoplasia and other congenital defects (AR)

Multiple non-erupting permanent teeth associated with delayed eruption of deciduous teeth, midface hypoplasia, abnormalities of the external ear, and genua valga. The unerupted teeth appear to be ankylosed, and have malformed roots, hypercementosis and many cementicles in the periodontal membrane.

McK 27305

Stoelinga, P.J.W., DeKoomen, H.A. & Davis, G.B. (1976) Multiple non-erupting teeth, maxillo-zygomatical hypoplasia and other congenital defects: an autosomal recessive disorder. *Clinical Genetics*, **10**, 222–225.

***IV.I.14.* (*I.C.6.*) *Osteopetrosis* (*'marble bones'; Albers–Schönberg disease*)** (H)

See I.C.6.

IV.I.15. Progeria (AR)

A combination of dwarfism and precocious senility. By ten years of age, height is approximately that of a normal three-year-old child. The face is disproportionately small, giving the head a hydrocephalic appearance, though no hydrocephaly is present and the head is actually smaller than normal. The skin is thin and atrophic. Scalp hair and eyebrows are lost at about one year of age and replaced by a downy fuzz. Delayed eruption has been reported in most cases, and the deciduous dentition is often retained.

McK 26410 *SHV* 622–625

Album, M.M. & Hope, J.W. (1958) Progeria. Report of a case. *Oral Surgery*, **11**, 985–998.
Gabr, M. (1954) Progeria: review of the literature with report of a case. *Archives of Pediatrics*, **71**, 35–46.
Ozonoff, M.B. & Clemett, A.R. (1967) Progressive osteolysis in progeria. *American Journal of Roentgenology*, **100**, 75–79.

***IV.I.16.* (*IV.D.18.*) *Pseudohypoparathyroidism* (*PHP; Albright hereditary osteodystrophy*)** (XL or AD)

See IV.D.18.

IV.I.17.* (*I.C.8.*) *Pyknodysostosis (AR*)

See I.C.8.

IV.I.18.* (*I.D.11., II.C.1.*) *Rutherfurd syndrome (AD*)

See II.C.1.

IV.J. Disorders in which There may be Natal Teeth

***IV.J.1.* (*II.A.2., IV.A.2., IV.D.3.*) *Chondroectodermal dysplasia* (*Ellis–van Creveld syndrome*)** (AR*)

See II.A.2.

***IV.J.2.* (*IV.A.7.*) *Hallermann–Streiff syndrome* (*oculomandibulodyscephaly*)** (AR)

A syndrome of beaked nose, mandibular and sometimes malar hypoplasia, dwarfism, hypotrichosis, microphthalmia, blue scleras, cataract, and a variably shaped, sometimes bulging, skull. Teeth may be absent or malformed. There may also be supernumerary teeth, and natal teeth have been reported in a number of cases.

McK 23410 *OFG* 138, 143, 606 *SHV* 557–561

Falls, H.F. & Schull, W.J. (1960) Hallermann–Streiff syndrome: a dyscephaly with congenital cataracts and hypotrichosis. *Archives of Ophthalmology*, **63**, 409–420.
Hoefnagel, D. & Benirschke, K. (1965) Dyscephalia mandibulo-oculo-facialis (Hallermann–Streiff syndrome). *Archives of Disease in Childhood*, **40**, 57–61.
Hutchinson, M. (1971) Oral manifestations of oculomandibulodyscephaly with hypotrichosis (Hallermann–Streiff syndrome). *Oral Surgery*, **31**, 234–244.

IV.J.3. Natal teeth (AD)

The presence of teeth at birth, unassociated with major malformations.

McK 18705

Sibert, J.R. & Porteous, J.R. (1974) Erupted teeth in the newborn. 6 members of a family. *Archives of Disease in Childhood*, **49**, 492–493.

IV.J.4.* (*III.A.5.*) *Pachyonychia congenita (AD*)

See III.A.5.

SINGLE GENE DISORDERS WITH FUNCTIONAL OR NEUROLOGICAL MANIFESTATIONS

V.A. Disorders Affecting the Temporomandibular Joint and/or Mandibular Movement

V.A.1. (III.F.1., IV.D.6., IV.H.1.) Ehlers–Danlos syndromes (H)

See III.F.1.

V.A.2. Hereditary quivering of the chin (AD*)

A transient tremor of the mentalis muscle triggered by emotional disturbances and/or specific stimuli, both pleasant and unpleasant. The tremor disappears during sleep, and attacks become less frequent with age.

McK 19010 *SHV* 208–209

Grossman, B.J. (1957) Trembling of the chin–an inheritable dominant character. *Pediatrics*, **19**, 453–455.

Laurance, B.M., Matthews, W.B. & Diggle, J.H. (1968) Hereditary quivering of the chin. *Archives of Disease in Childhood*, **43**, 249–251.

Wadlington, W.B. (1958) Familial trembling of the chin. *Journal of Pediatrics*, **53**, 316–321.

V.A.3. Inability to open the mouth fully (AD*)

A disorder in which inability to open the mouth fully is associated with pseudocamptodactyly (curvature of the fingers at all interphalangeal joints on dorsiflexion of the wrist), and moderately short stature. There may also be other minor musculoskeletal abnormalities. The maximum distance recorded between upper and lower incisal edges is 1.8 cm. This limitation of mouth opening has been shown to be due to an abnormally large coronoid process of the mandible that butts against the posterior surface of the maxillary zygomatic process when opening is attempted, preventing further rotation of the mandible.

McK 15830 *SHV* 390–392

De Jong, J.G.Y. (1971) A family showing strongly reduced ability to open the mouth and limitation of some movements of the extremities. *Humangenetik*, **13**, 210–217.

Hecht, F. & Beals, R.K. (1969) Inability to open the mouth fully: an autosomal dominant phenotype with facultative campylodactyly and short stature. *Birth Defects*, **5**(3), 96–98.

Horowitz, S.L., McNulty, E.C. & Chabora, A.J. (1973) Limited intermaxillary opening–an inherited trait. *Oral Surgery*, **36**, 490–492.

Mabry, C.C., Barnett, I.S., Hutcheson, M.W. & Sorenson, H.W. (1974) Trismus-pseudocamptodactyly syndrome. *Journal of Pediatrics*, **85**, 503–508.

Wilson, R.V., Gains, D.L., Brooks, A., Carter, T.S. & Nance, W.E. (1969) Autosomal dominant inheritance of shortening of the flexor profundus muscle-tendon unit with limitation of jaw excursion. *Birth Defects*, **5**(3), 99–102.

V.A.4. Ophthalmo-mandibulo-melic dysplasia (AD*)

Temporomandibular joint fusion, absent coronoid process and obtuse mandibular angle, associated with corneal opacities, and short forearms due to radio-humeral and radio-ulnar dislocations with aplasia of the lateral humeral condyle, head of radius and distal third of ulna.

McK 16490 *SHV* 737

Pillay, V.K. (1964) Ophthalmo-mandibulo-melic dysplasia, an hereditary syndrome. *Journal of Bone and Joint Surgery*, **46A**, 858–862.

V.B. Disorders with Primarily Neurological Manifestations

V.B.1. (II.B.2.) Familial dysautonomia (Riley–Day syndrome) (AR*)

A disorder, primarily of the autonomic nervous system, in which there is reduced lacrimation, vasomotor instability, paroxysmal hypertension, excessive sweating and drooling. In addition there is relative insensitivity to pain and to taste, and frequently a diminished gag reflex or disturbance of swallowing. Lingual fungiform and circumvallate papillae may be reduced in number or absent. Speech is often slurred.

McK 22390 *OFG* 300, 351–353 *SHV* 300–305

Brunt, P.W. & McKusick, V.A. (1970) Familial dysautonomia: a report of genetic and clinical studies with a review of the literature. *Medicine*, **49**, 343–374.

Henkin, R. & Kopin, I. (1964) Abnormalities of taste and smell thresholds in familial dysautonomia: improvement with methacholine. *Life Sciences*, **3**, 1319–1325.

Pearson, J., Finegold, M.J. & Budzilovich, G. (1970) The tongue and taste in familial dysautonomia. *Pediatrics*, **45**, 739–745.

Reitman, A.A., Blacharsh, C. & Levy, J.M. (1965) Clinical evaluation of the dental aspects of familial dysautonomia. *Journal of the American Dental Association*, **71**, 1436–1446.

Smith, A.A., Farbman, A. & Dancis, J. (1965) Tongue in familial dysautonomia. *American Journal of Diseases of Children*, **110**, 152–153.

V.B.2. Inherited sensory neuropathies (H)

A group of disorders, some of which show widespread insensitivity to pain. There is consequent self-mutilation, often particularly evident in the tongue and lips, and no toothache, even in the presence of an apical abscess.

McK 24300 (AR*), 25675 (AR*), 25680 (AR*), 25690 (AR*) *OFG* 300–302 *SHV* 188–191

Murray, T.J. (1973) Congenital sensory neuropathy. *Brain*, **96**, 387–394.
Ogden, T.E., Robert, F. & Carmichael, E.A. (1959) Some sensory syndromes in children: indifference to pain and sensory neuropathy. *Journal of Neurology, Neurosurgery and Psychiatry*, **22**, 267–276.
Thrush, D.C. (1973) Congenital insensitivity to pain. *Brain*, **96**, 369–386.
Vassella, F., Emrich, H.M., Kraus-Ruppert, R., Aufdermaur, F. & Tönz, O. (1968) Congenital sensory neuropathy with anhidrosis. *Archives of Disease in Childhood*, **43**, 124–130.

V.B.3. Lesch–Nyhan syndrome (*HGPRTase deficiency*) (XL*)

A syndrome of mental retardation, spastic cerebral palsy, choreoathetosis, excessively high serum uric acid levels, and compulsive self-destructive biting of the fingers and lips. In contrast to the inherited sensory neuropathies where there is self-mutilating behaviour (see V.B.2.), no impairment of pain perception can be demonstrated. The disorder is due to a deficiency of the enzyme hypoxanthine–guanine phosphoribosyl transferase (HGPRT).

MBID 1011–1036 *McK* 30800 *OFG* 399–400 *SHN* 433–435

Nyhan, W.L. (1972) Clinical features of the Lesch-Nyhan syndrome. *Archives of Internal Medicine*, **130**, 186–192.
Reed, W.B. & Fish, C.H. (1966) Hyperuricemia with self mutilation and choreoathetosis. *Archives of Dermatology*, **94**, 194–195.

V.B.4. (II.B.3.) Melkersson syndrome (Melkersson–Rosenthal syndrome) (AD*)

Facial paralysis, indistinguishable from Bell's palsy, frequently combined with facial oedema and fissured tongue. Recurrent attacks of facial oedema, especially of the upper lip, usually start during childhood prior to the onset of facial paralysis. Facial palsy appears suddenly before 20 years of age, and, though ultimate recovery is usual, relapses often occur.

McK 15590 *SHN* 468–472

Burzynski, N.J. & Weisskopf, B. (1973) Familial occurrence of Bell's palsy. *Oral Surgery*, **36**, 504–506.
Nally, F.F. (1970) Melkersson–Rosenthal syndrome. *Oral Surgery*, **29**, 694–703.

V.B.5. (II.B.4.) Moebius syndrome (congenital facial diplegia) (AD*)

A combination of sixth and seventh cranial nerve palsies, present from birth, sometimes associated with palsies of other cranial nerves, limb dysplasia, unilateral or bilateral tongue hypoplasia, and a variety of other defects.

McK 15790 *SHN* 575–578

Becker-Christensen, F. & Lund, H.T. (1974) A family with Möbius' syndrome. *Journal of Pediatrics*, **84**, 115–117.
Evans, P.R. (1955) Nuclear agenesis. Möbius' syndrome: the congenital facial diplegia syndrome. *Archives of Disease in Childhood*, **30**, 237–243.
Gutman, D., Sharon, A. & Laufer, D. (1973) Moebius syndrome. *British Journal of Oral Surgery*, **11**, 20–24.

INDEX OF SINGLE GENE DISORDERS

The following is an index of single gene disorders that can be found in the catalogue, including some of their more common alternative names. If the biochemical basis of a disorder is understood this is indicated by (*b*). For disorders that appear more than once in the classification, the first catalogue number given refers to the position where a brief description and references can be found. Second and subsequent catalogue numbers (given in brackets) refer only to other positions in the classification and are not sources of additional information.

Focal dermal hypoplasia (Goltz syndrome), III.C.3. (IV.A.6., IV.D.9., IV.I.9.)
Follicular keratosis – *see* Darier–White disease
Freeman–Sheldon syndrome – *see* Craniocarpotarsal dysplasia
Gardner syndrome (intestinal polyposis III), I.C.1. (IV.E.3.)
Gaucher disease (*b*), I.D.5.
Generalized cortical hyperostosis (hyperphosphatasia tarda; Van Buchem disease), I.C.2.
Gingival fibromatosis in juvenile hyaline fibromatosis (Murray–Puretić–Drescher syndrome), II.C.1.
Gingival fibromatosis in the Rutherfurd syndrome, II.C.1. (I.D.11., IV.I.18.)
Gingival fibromatosis with ear, nose, bone, nail defects and splenomegaly (Laband syndrome), II.C.1.
Gingival fibromatosis with hypertrichosis, II.C.1.
Gingival fibromatosis with microphthalmia, mental retardation, athetosis and hypopigmentation (Cross syndrome; oculo-cerebral syndrome with hypopigmentation), II.C.1.
Gingival fibromatosis with progressive deafness, II.C.1.
Gingival fibromatosis without hypertrichosis, II.C.1.
Globodontia – *see* Otodental dysplasia
Goltz syndrome – *see* Focal dermal hypoplasia
Gorlin syndrome – *see* Basal cell naevus syndrome
Gottlieb syndrome – *see* Periodontosis
Haemoglobinopathies (*b*), I.D.6. (IV.G.6.)
Hailey–Hailey disease – *see* Familial benign chronic pemphigus
Hallerman–Streiff syndrome (oculomandibulodyscephaly), IV.J.2. (IV.A.7.)
Hereditary arthro-ophthalmopathy with retinal detachment and cleft palate – *see* Stickler syndrome
Hereditary benign intraepithelial dyskeratosis, III.A.4.
Hereditary generalized microdontia, IV.A.8.
Hereditary haemorrhagic telangiectasia, III.E.3.
Hereditary opalescent dentine – *see* Dentinogenesis imperfecta, type II
Hereditary quivering of the chin, V.A.2.
Hunter syndrome – *see* Mucopolysaccharidosis II
Hurler syndrome – *see* Mucopolysaccharidosis I–H
Hyalinosis cutis et mucosae – *see* Lipoid proteinosis
Hyperoxaluria – *see* Oxalosis
Hyperphosphatasia tarda – *see* Generalized cortical hyperostosis
Hypodontia and nail dysplasia (tooth and nail syndrome), IV.A.9.
Hypodontia, taurodontism and sparse scalp hair, IV.A.10.
Hypohidrosis, thin wiry hair, dystrophic nails and cleft lip-palate, I.A.12.
Hypohidrotic (anhidrotic) ectodermal dysplasia, IV.A.11.
Hypophosphataemia (vitamin D-resistant rickets), IV.D.10.
Hypophosphatasia (*b*), IV.D.11. (IV.E.4., IV.H.2.)
Hypoxanthine guanine phosphoribosyl transferase (HGPRTase) deficiency – *see* Lesch–Nyhan syndrome
I-cell disease – *see* Mucolipidosis II
Inability to open the mouth fully, V.A.3.
Incontinentia pigmenti, IV.A.12. (IV.I.10.)
Infantile cortical hyperostosis (Caffey disease), I.C.3.
Inherited sensory neuropathies, V.B.2.
Intestinal polyposis II – *see* Peutz–Jeghers syndrome
Intestinal polyposis III – *see* Gardner syndrome
Juvenile cortical hyperostosis (congenital hyperphosphatasia; juvenile Paget disease), I.C.4.
Juvenile hyaline fibromatosis – *see* Gingival fibromatosis in juvenile hyaline fibromatosis
Juvenile Paget disease – *see* Juvenile cortical hyperostosis
Laband syndrome – *see* Gingival fibromatosis with ear, nose, bone, nail defects and splenomegaly
Lacrimo-auriculo-dento-digital (LADD) syndrome, IV.D.12.
Larsen syndrome (multiple congenital dislocations, flattened facies and cleft palate), I.B.5.
Lesch–Nyhan syndrome (hypoxanthine guanine phosphoribosyl transferase–HGPRTase–deficiency) (*b*), V.B.3.
Lipoid proteinosis (hyalinosis cutis et mucosae), III.B.1. (IV.A.13., IV.D.13.)
Mandibulofacial dysostosis (Treacher Collins syndrome), I.B.6
'Marble bones' – *see* Osteopetrosis
Marfan syndrome, I.D.7.
Meckel syndrome, I.B.7.
Melkersson syndrome (Melkersson–Rosenthal syndrome), V.B.4. (II.B.3.)
Melkersson–Rosenthal syndrome – *see* Melkersson syndrome
Metachromatic leucodystrophy (*b*), IV.G.2.
Mibelli disease – *see* Porokeratosis
Moebius syndrome (congenital facial diplegia), V.B.5. (II.B.4.)
Mohr syndrome – *see* Oral-facial-digital syndrome II
Molar reinclusion, IV.I.11.
Morquio disease – *see* Mucopolysaccharidosis IV
Mucolipidosis II (I-cell disease), II.C.2. (IV.D.14.)
Mucopolysaccharidosis I–H (Hurler syndrome) (*b*), I.D.8. (II.A.6., II.B.5., II.C.3., IV.G.3., IV.I.12.)
Mucopolysaccharidosis II (Hunter syndrome) (*b*), I.D.9. (II.A.7., II.B.6.)
Mucopolysaccharidosis IV (Morquio disease) (*b*), IV.D.15.
Multiple congenital dislocations, flattened facies and cleft palate – *see* Larsen syndrome
Multiple hamartoma and neoplasia syndrome (Cowden syndrome), III.C.4.
Multiple mucosal neuromas, phaeochromocytoma and medullary thyroid carcinoma, III.B.2.
Multiple non-erupting teeth, maxillo-zygomatic hypoplasia and other congenital defects, IV.I.13. (IV.E.5.)
Multiple odontomas, I.C.5.
Multiple odontomas, oesophageal stenosis and chronic interstitial cirrhosis of the liver, I.C.5.
Murray–Puretić–Drescher syndrome – *see* Gingival fibromatosis in juvenile hyaline fibromatosis
Myeloperoxidase deficiency (*b*), III.H.6.
Natal teeth, IV.J.3.
Neurofibromatosis (Von Recklinghausen disease), III.B.3. (I.D.10.)
Niemann–Pick disease (sphingomyelin lipidosis), (*b*), IV.G.4.

Oculo-cerebral syndrome with hypopigmentation – *see* Gingival fibromatosis with microphthalmia, mental retardation, athetosis and hypopigmentation
Oculo-dento-digital syndrome – *see* Oculo-dento-osseous dysplasia
Oculo-dento-osseous dysplasia (oculo-dento-digital syndrome), IV.D.16.
Oculomandibulodyscephaly – *see* Hallermann–Streiff syndrome
Ophthalmo-mandibulo-melic dysplasia, V.A.4.
Oral–facial–digital syndrome I (OFD I syndrome), I.A.13. (II.A.8., II.B.7., IV.A.14.)
Oral–facial–digital syndrome II (Mohr syndrome; OFD II syndrome), II.B.8. (II.A.9.)
Osteitis deformans – *see* Paget disease
Osteogenesis imperfecta – *see* Dentinogenesis imperfecta, type I
Osteopetrosis (Albers–Schönberg disease; 'marble bones'), I.C.6. (IV.I.14.)
Otodental dysplasia (globodontia), IV.A.15.
Oto-palato-digital syndrome (OPD syndrome), I.A.14.
Oxalosis (hyperoxaluria) (*b*), IV.F.2. (IV.G.5.)
Pachyonychia congenita, III.A.5. (IV.J.4.)
Paget disease of bone (osteitis deformans), I.C.7. (IV.E.6.)
Palmoplantar hyperkeratosis and attached gingival hyperkeratosis, II.C.4.
Palmoplantar hyperkeratosis and periodontoclasia (Papillon–Lefèvre syndrome), IV.H.3.
Papillon–Lefèvre syndrome – *see* Palmoplantar hyperkeratosis and periodontoclasia
Periodontosis (familial juvenile periodontosis; Gottlieb syndrome), IV.H.4.
Peutz–Jeghers syndrome (intestinal polyposis II), II.A.10. (III.C.5.)
Pierre Robin syndrome – *see* Robin anomalad
Popliteal pterygium syndrome – *see* Cleft lip-palate, mucous cysts of the lower lip, popliteal pterygium, digital and genital anomalies
Porokeratosis (Mibelli disease), III.A.6.
Porphyria (*b*), IV.F.3. (III.D.5., IV.H.5.)
Premolar aplasia, hyperhidrosis and canities prematura (Böök syndrome; PHC syndrome), IV.A.16.
Progeria, IV.I.15.
Pseudohypoparathyroidism (Albright hereditary osteodystrophy; PHP), IV.D.18. (IV.I.16.)
Pseudoxanthoma elasticum, III.B.4.
Pterygium syndrome, I.A.15.
Pulpal dysplasia – *see* Dentine dysplasia, type II
Pyknodysostosis, I.C.8. (IV.I.17.)
Pyramidal molar roots, juvenile glaucoma and unusual morphology of upper lip (Ackerman syndrome), IV.A.17.
Radicular dentine dysplasia – *see* Dentine dysplasia, type I)
Rieger syndrome, IV.A.18.
Riley–Day syndrome – *see* Familial dysautonomia
Roberts syndrome (Appelt syndrome; cleft lip-palate and tetraphocomelia), I.A.16.
Robin anomalad (cleft palate, micrognathia and glossoptosis; Pierre Robin syndrome), I.A.17.
Rutherfurd syndrome, II.C.1. (I.D.11., IV.I.18.)
Sensory neuropathies – *see* Inherited sensory neuropathies
Sclerosteosis (cortical hyperostosis with syndactyly), I.C.9.
Shell teeth – *see* Dentinogenesis imperfecta, types II and III
Sickle cell anaemia – *see* Haemoglobinopathies
Sphingomyelin lipidosis – *see* Niemann–Pick disease
Stickler syndrome (hereditary arthro-ophthalmopathy with retinal detachment and cleft palate), I.B.8.
Tooth and nail syndrome – *see* Hypodontia and nail dysplasia
Total absence of permanent teeth, IV.A.19.
Treacher Collins syndrome – *see* Mandibulofacial dysostosis
Tricho-dento-osseous syndrome – *see* Amelogenesis imperfecta with taurodontism, curly hair and sclerotic bones
Tuberous sclerosis, III.B.5. (I.C.10., I.D.12., IV.D.19.)
Universal permanent alopecia, psychomotor epilepsy, pyorrhoea and mental subnormality, IV.H.6.
Van Buchem disease – *see* Generalized cortical hyperostosis
Vitamin D-dependent rickets, IV.D.20.
Vitamin D-resistant rickets – *see* Hypophosphataemia
Von Recklinghausen disease – *see* Neurofibromatosis
Whistling face syndrome – *see* Craniocarpotarsal dysplasia
White folded dysplasia of the mucous membranes – *see* White sponge naevus of Cannon
White sponge naevus of Cannon (white folded dysplasia of the mucous membranes), III.A.7.
X-linked cleft palate, I.A.18.
Xanthomatosis (*b*), III.B.6.

CHAPTER 2

Biological Variation and its Measurement

Variation is a fundamental characteristic of all biological processes, and it is only through a study of variation and its underlying causes that insight can be gained into the ways in which biological systems operate. This chapter is concerned with types and sources of variation, where variation occurs, and some elementary statistical ideas that can be used in the interpretation of observed differences.

VARIATION AND WHERE IT COMES FROM

Variation can be categorized according to the nature of the differences that are observed, and also according to the underlying basis for these differences.

Types of variation

The majority of biological variation falls into one of the following four categories: discrete, continuous, quasicontinuous or discontinuous variation.

Characters that show discrete variation exist in two or more qualitatively different forms. Those existing in two forms only, such as sex, are known as dimorphisms, and those for which there are more than two forms, such as ABO blood type, are known as polymorphisms. Using blood type as an example, individuals are either of one type or another, there is no continuum of intermediates, no variation within each type and no continuous scale of measurement against which different types can be compared. Discrete variation between individuals usually has a simple genetic basis, different forms of the same character being produced by different alleles at the same locus (see Book 2, Chapter 2).

Continuous variables, such as height, weight, tooth size, rate of tooth eruption or the activity of an enzyme, are characters that can be measured against an appropriate continuous scale. Continuous variation is quantitative rather than qualitative, and levels of expression of the same continuously variable character in different situations can be compared by means of the common scale of measurement. Continuous variation usually has a multifactorial basis, several genes and environmental influences, each with a relatively small effect, contributing to the level of expression.

Characters that are either present or absent, but when present vary continuously, are called quasicontinuous variables (or threshold characters). An example is the accessory feature on the mesiolingual surface of the crown of upper molars, known as the cusp of Carabelli (or Carabelli's trait). It is present on some teeth but not on others, and when present it may appear as anything from a small pit or groove to a pronounced extra cusp. The accepted explanation of quasicontinuous variation rests on the assumption that there is an underlying scale of continuous variation of some attribute (the result of a combination of all the genetic and environmental factors involved) that is immediately related to the development of the character. The character is absent in situations where the level on the scale fails to reach a critical threshold value, and present when the level exceeds this threshold value. The greater the distance above the threshold the more intense is the expression of the character. A quasicontinuous character can therefore be regarded as a continuous variable whose expression has a 'visible' and a 'nonvisible' range. Quasicontinuous variation, like continuous variation, usually has a multifactorial basis.

Variation in number of similar items is discontinuous in that the number of items must always be an integer (whole number); for example, the number of teeth an individual possesses or the number of children in a family. Variation occurs about a modal number, the number that is found more frequently than any other, but only in decreasing or increasing integer steps. For

example, the modal number of teeth in a fully developed human permanent dentition is thirty-two. In other words, individuals with thirty-two teeth make up the largest category. Other individuals have thirty-one, thirty or even fewer teeth, and still others have thirty-three, thirty-four and sometimes even more. Development never produces, for instance, thirty-one and a half teeth. Each structure formed is a whole tooth even though teeth vary continuously in size. It is sometimes useful to think of discontinuous variation as an extension of quasicontinuous variation, with the underlying scale divided by several thresholds, each threshold separating one integer value from the next. Discontinuous variation, like continuous and quasicontinuous variation, usually has a multifactorial basis.

Sources of variation

Differences may occur for either genetic or environmental reasons, or some combination of both. Observed variation is also contributed to by errors of observation or measurement and by differences due to chance. However, even if all the observed variation can reasonably be attributed to measurement error and chance, undisclosed biological differences may still exist.

HEREDITY AND ENVIRONMENT

The variation observed among living things is composed of hereditary and environmental components. Heredity supplies the potential and the environment determines how this potential is expressed. Variation may also be broken down into other causal components, either subdivisions of heredity or environment, or components that contain both hereditary and environmental fractions.

Every individual, with the exception of identical twins, has a unique genetic constitution. There is therefore enormous genetic diversity, and this is partly responsible for the observed differences between individuals. Differences between 'normal' individuals may be discrete differences under simple genetic control (as in the case of blood type); or multifactorially controlled differences leading to continuous variation (as for body height), quasicontinuous variation (as for a dental morphological variant), or discontinuous variation (as for tooth number). Gross differences from the population norm may be caused by single genes with major effects or by chromosomal abnormalities. For example, in achondroplasia, an abnormality of cartilage controlled by a single gene, there is reduced epiphyseal growth resulting in dwarf stature. Achondroplastics fall well outside the normal range of body height. In Down's syndrome (mongolism) several abnormalities, from the characteristic facial appearance to mental retardation and congenital heart defects, stem from the presence of one small additional chromosome, or sometimes only part of it.

One of the major subcomponents of normal genetic variation is the difference between the sexes, a difference that ultimately rests on the different sex chromosome complements (XY and XX) of males and females. In addition to the more obvious differences between the sexes there are less common but nevertheless interesting ones, particularly in the expression of certain developmental and metabolic abnormalities. For example, cleft lip with or without a cleft of the palate affects males more frequently than females (about 60% of cases are males), and also tends to be more severe in males than in females. A more extreme difference between the sexes is found in congenital dislocation of the hip, where there are about six times as many female cases as male cases. A good example of a metabolic disorder affecting the sexes differently is gout, where the afflicted are predominantly males.

All individuals are, of necessity, exposed to at least slightly different environments, simply because more than one individual cannot be in exactly the same place at the same time. Extreme environmental differences of, for example, climate, altitude or availability of food, are understandably likely to have definite biological effects, but even minor environmental variation within a population occupying a restricted area often contributes considerably to observed differences between individuals. Environmental effects are superimposed on genetic differences. Thus individuals with the same hereditary potential may grow to different heights, depending on the environments to which they have been exposed during the growth period. Similarly, individuals with different hereditary potentials may grow to the same height if an environmental difference exactly compensates for the hereditary one. Some ways of estimating the relative contributions of genetic and environmental differences to the observed variation between individuals are described elsewhere (Book 2, Chapter 2).

For most characters studied, members of the same family tend to be more alike than unrelated individuals. This resemblance between relatives can usually be attributed largely to the fact that relatives have a proportion of their genes in common. However, particularly for characters showing continuous, quasicontinuous or discontinuous variation, the common environment experienced by members of the same family may also contribute to the resemblance between relatives. Members of a family group may therefore be alike not only because they have inherited the same genes but also because they are exposed to similar environmental influences.

For some characters a given change in the environment produces different observed effects in individuals with different genetic constitutions. Many examples of this kind of interaction between heredity and environment are found in the field of disease susceptibility. There may be inherited variation of susceptibility to a disease produced by a specific extrinsic factor, for example a particular microorganism, making individuals with different inherited susceptibilities react differently to the same level of the same pathogenic agent.

MEASUREMENT ERROR AND CHANCE VARIATION

Most measuring procedures are not absolutely accurate; that is, measurements made of an identical situation on more than one occasion may give slightly different answers. Some proportion of the variation that is recorded by measurement can therefore arise from measurement error. The importance of the error in a given situation can be assessed by measuring the same set of items on two occasions and by comparing the differences between first and second sets of measurements with the variation within each set of measurements. If the differences between first and second measurements are very small compared with the variation between one item and the next, measurement error can be ignored.

In addition to the differences ascribable to major or minor inherited or environmental differences or to measurement error there is always a small residual component of variation due to chance. This is the variation that would be recorded, if it were possible to do so, between genetically identical individuals in identical environments with a perfect measurement technique. It is the result of random variation of cell function and interaction.

HETEROGENEITY

Absence of observed variation, other than that attributable to measurement error and to chance, does not imply absence of variation at a more fundamental level. Different combinations of hereditary and environmental factors may produce the same end result, so a group of individuals with, for example, a particular developmental malformation may be heterogeneous; that is, what appears to be the same condition may have been produced in a number of different ways. Any attempt to analyze such a group as a whole can only lead to misleading or nonsensical conclusions. Every effort should be made to subdivide it into more homogeneous classes using whatever information is available. For instance, clefts of the palate may occur either in association with cleft lip (cleft lip with or without a cleft of the palate, CL(P)), or alone (isolated cleft palate, CP). When studied further, these two classes of cleft palate cases are found to differ in other respects also. First, different developmental processes are involved. Cleft palate in CP cases results directly from an abnormality at the stage of secondary palate (hard palate) formation, whereas in CL(P) cases it is a consequence of failure of fusion of the primary palate (lip and alveolus) at an earlier stage of development. Second, many CL(P) cases have affected relatives but fewer CP cases do; and affected relatives of CL(P) cases have CL(P) not CP. There are therefore grounds for believing that CL(P) is a separate entity from CP and has a larger genetic component in its aetiology.

WHERE VARIATION OCCURS

Differences exist not only between individuals but also within individuals, between tissues or regions of the body, and with time. Differences are also found between populations or groups of individuals.

Variation within individuals

All the somatic cells of an individual contain, at some stage, the same complement of genes, yet differences within an individual begin to appear early in development when cells or groups of cells start to differentiate along a variety of developmental pathways. The basis for this differentiation seems to be that only particular

genes are 'switched on' in particular cells, the remaining majority of genes in each cell type being inactive for most of the time. Discrete differences between tissues can be detected by immunological techniques, each tissue type having a unique set of chemical specificities that is presumably a direct consequence of the activity of a particular set of genes. These discrete differences may be expressed at a different level through variation in such characters as cell size, shape and staining intensity, characters that can be measured on continuous scales.

A second kind of variation that occurs within individuals is that between right and left sides of the body. In bilaterally symmetrical organisms many structures are represented on both sides and develop as mirror images of each other. However, perfect symmetry is rarely attained, minor differences between sides being the general rule. It is reasonable to assume, barring exceptional circumstances, that at any given stage of development the same genes are active in the same tissues on both sides of the body. Failure of bilaterally represented structures to form as exact mirror images of each other is therefore an expression of imprecise genetic control over development.

Variation within individuals may also occur with time. There may be changing levels of expression of a character, either over the growth period or from day to day due to cyclic changes of physiology. For instance, there is the continuous change in body height through infancy, childhood and adolescence into adulthood, and diurnal variation throughout life caused by postural fatigue, individuals tending to be taller in the morning and shorter in the evening. Parallel instances can be found within developing systems. During tooth formation, for example, cells of the internal enamel epithelium become progressively more columnar as they differentiate into ameloblasts, reaching a maximum height when enamel formation is in progress. A continued rhythmic variation of ameloblast function then occurs as enamel matrix is being laid down, variation that results in the incremental lines (brown striae) of Retzius, visible in sections of fully formed enamel.

Variation between individuals and between groups of individuals

DISCRETE VARIATION

Discrete variation between individuals means that some individuals show one form of a character whereas others show other forms. Differences between groups are measured in terms of relative frequencies of the different forms. For example, there is variation both within and between racial groups for ABO blood type. About 45% of caucasians are of blood type A, whereas only 28–29% of individuals from mongoloid or negro groups are of blood type A (Table 2.1).

Table 2.1. Percentage frequencies of ABO blood types in large samples from different racial groups.

	A	B	AB	O
Caucasian (North Europe)	45	8	3	44
Negro (Africa)	29	17	4	50
Mongoloid (China)	28	24	7	41

CONTINUOUS VARIATION

Continuous variation results in individuals occupying different positions on the scale of measurement. In any range on any scale there is theoretically an infinite number of possible values, but for practical purposes measurements are made in terms of a chosen size of subdivision. A population is described by the number of individuals falling within each subdivision; that is, by the distribution of individuals over these subdivisions of the scale. Examples of two such distributions are shown in Fig 2.1 a and b. They are distributions of third molar size in two genetically different groups of mice, measured to the nearest hundredth of a millimetre. The horizontal scale is marked off in the chosen subdivisions and the vertical scale shows how many teeth fell into each one. The pictures shown in Fig. 2.1 a and b are called histograms.

Each of these histograms approaches the most commonly encountered type of distribution for continuous variables, the normal distribution, whose 'ideal' shape is described by the bell-shaped normal curve. Normal curves corresponding to the two histograms are shown in Fig. 2.1 c and d. The horizontal scale for these curves, though still a scale of tooth size, is not subdivided, and the vertical scale measures the relative frequency with which teeth of particular sizes are found in each 'idealized' group. These 'ideal' curves can be regarded as histograms that would be produced if the groups were infinitely large, and if tooth size were measured in infinitesimally small subdivisions. Other aspects of these distributions will be referred to later.

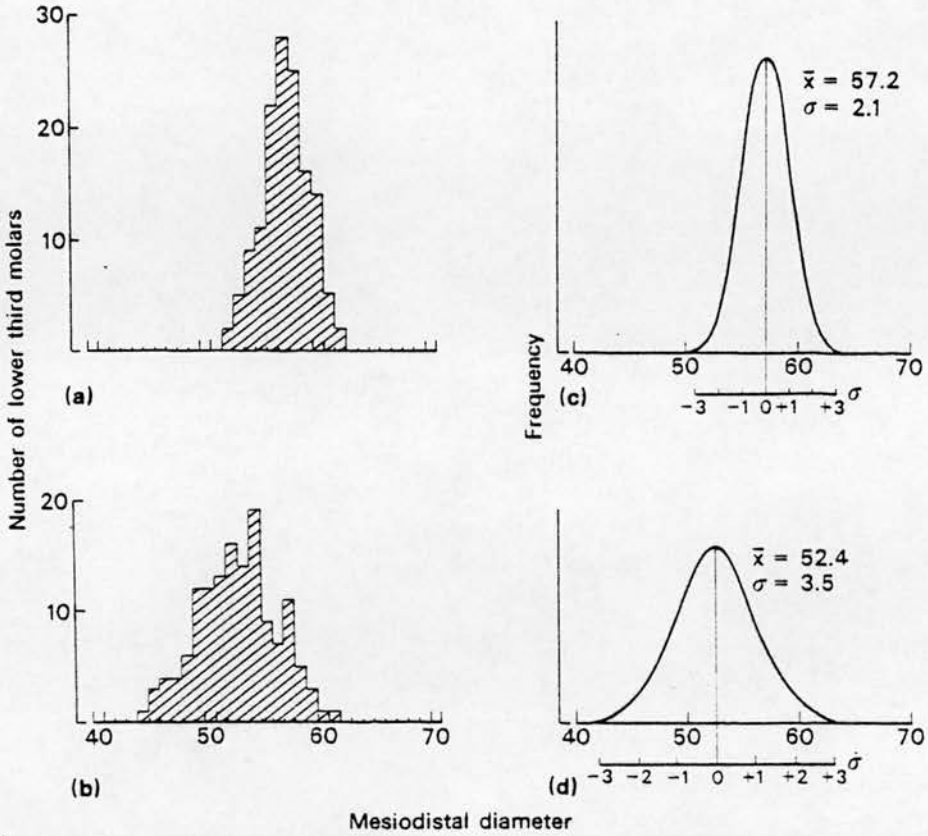


Fig. 2.1. Histograms showing the distribution of mesiodistal diameters of lower third molars in two groups of mice (a (n = 139) and b (n = 141)), and corresponding normal curves (c and d). 1 unit = 10 μm.

The normal curve has quite specific mathematical properties, and many statistical procedures are based on them. Before going into some of these properties a few symbols commonly used in the analysis of continuous variation must be introduced.

The average, or mean, of several measurements is equal to the sum of all the measurements divided by the number of measurements. If each measurement is given the symbol X, the sum of all the measurements can be represented by ΣX, where Σ (the Greek capital sigma) means 'sum of'. If there are n measurements altogether, and if $\bar{X}$ ('X-bar') stands for the mean of all these measurements, then $\bar{X} = \Sigma X/n$. The mean is an indication of the most fundamental property of a distribution, its position on the scale.

The second important property of a distribution is its spread on the scale. This can be expressed by the deviation of a 'typical' or 'standard' measurement from the mean of the distribution. The deviation of one measurement from the mean can be given the symbol x, so that $x = X - \bar{X}$. For measurements that fall above the mean x is positive, whereas for measurements that fall below the mean x is negative. It is a characteristic of the mean that Σx, the sum of all the deviations from it, is equal to zero. Therefore the average of all the deviations, which in other situations might be considered the 'typical' value, is also equal to zero and provides no information about the spread of the distribution on the scale. The way in which this difficulty is overcome is to take, instead of the deviations themselves, the squared deviations, which are all of necessity positive. The average of these squared deviations, known as the variance, is an indication of spread. However, since the quantity required here is an expression of deviation (rather than squared deviation) the square

root of the variance is used. This is known as the standard deviation and is given the symbol σ (the Greek lower case sigma). Thus, $\sigma = \sqrt{\Sigma x^2/n}$. Table 2.2 illustrates how a value for σ is calculated. The variance will be referred to again later, but for the present it should be noted that the variance is given by $\sigma^2 = \Sigma x^2/n$.

Table 2.2. Calculation of σ for ten lower third molar diameters (under X) from distribution b of Fig. 2.1. All values are in units of one hundredth of a millimetre.

X	$x = X - \bar{X}$	x^2
48	−4.7	22.09
54	+1.3	1.69
51	−1.7	2.89
58	+5.3	28.09
53	+0.3	0.09
50	−2.7	7.29
57	+4.3	18.49
54	+1.3	1.69
50	−2.7	7.29
52	−0.7	0.49
$\Sigma X = 527$	$\Sigma x = 0.0$	$\Sigma x^2 = 90.10$

$n = 10$
$\bar{X} = \Sigma X/n = 527/10 = 52.7$
$\sigma = \sqrt{\Sigma x^2/n} = \sqrt{90.1/10} = 3.0$

Consider the proportion of a normal distribution that falls within the range bounded by a given number of standard deviations, say w, on each side of the mean. If the mean is given the value zero on the scale this range extends from $-w\sigma$ to $+w\sigma$. As w increases, and therefore as the range on the scale increases, so does the proportion of the distribution falling within the range. It is a characteristic of the normal distribution that the area under the curve bounded by a given number of standard deviations on each side of the mean always contains the same proportion of the distribution, no matter what the degree of spread in terms of units on the scale of measurement (Table 2.3). One, two and three standard deviations on each side of the mean are shown for the two normal curves in Fig. 2.1. For each of these curves the area under the curve falling within the range -3σ to $+3\sigma$ amounts to 99.8% of the total (Table 2.3).

QUASICONTINUOUS VARIATION

With this in mind the third type of variation between individuals, quasicontinuous variation, can be considered in greater detail. In a population of individuals, some of whom show a quasicontinuous character and others of whom do not, the distribution on the underlying continuous scale is divided by the threshold. The shape of the whole distribution is therefore not fully disclosed, but unless there is reason to think otherwise it is often useful to assume it is normal. Fig. 2.2 (a and b) shows two histograms, the distributions of two groups of mice affected or nonaffected by the presence of a supernumerary cusp on the lower first molar. The zero category contains mice without the cusp and is separated from category 1 by the threshold. The arbitrarily defined categories 1 to 4 contain mice with progressively more extreme levels of expression of the cusp. In one of the groups most of the mice are affected. The histogram of this group (a) bears some resemblance to a normal distribution because most of the animals are allotted an appropriate value on the scale above the threshold. The histogram for the other group (b)

Table 2.3. The proportion of a normal distribution falling below, within and above the range bounded by different numbers of standard deviations from the mean. The ranges are expressed relative to a mean at position zero (see, for example, standard deviation scales in Fig. 2.1 c and d).

	Percentage of distribution			
Range	Below range	Within range	Above range	Total outside range
$-0.5\sigma - +0.5\sigma$	31.0	38.0	31.0	62.0
$-1.0\sigma - +1.0\sigma$	15.9	68.2	15.9	31.8
$-1.5\sigma - +1.5\sigma$	6.6	86.8	6.6	13.2
$-2.0\sigma - +2.0\sigma$	2.3	95.4	2.3	4.6
$-2.5\sigma - +2.5\sigma$	0.6	98.8	0.6	1.2
$-3.0\sigma - +3.0\sigma$	0.1	99.8	0.1	0.2

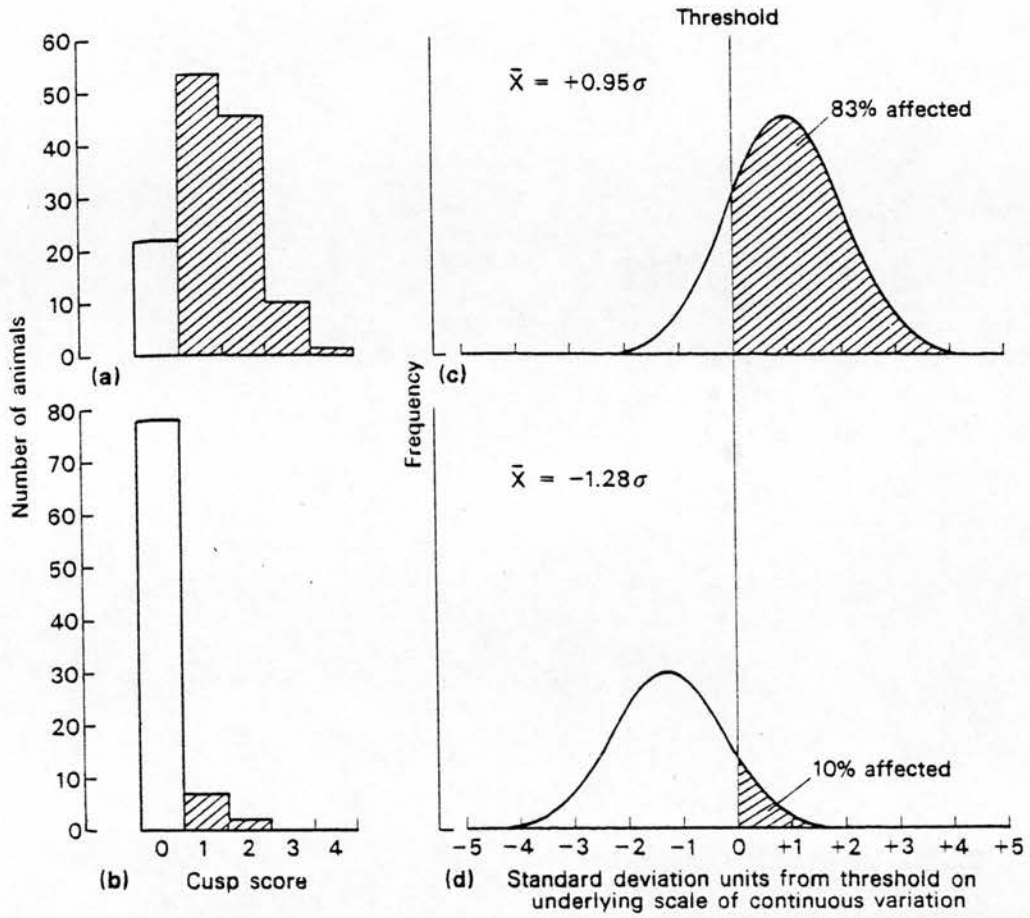


Fig. 2.2. Histograms showing the distribution of scores for a supernumerary cusp on the lower first molar in two groups of mice (a (n = 131) and b (n = 87)), and corresponding normal curves (c and d), assuming the same standard deviation for both groups. The shaded areas indicate affected and the non-shaded areas non-affected animals.

does not resemble a normal distribution because all unaffected mice, the majority in the group, are lumped together into the single zero category, even though they may occupy different positions below the threshold on the presumed underlying continuous scale. However, if it is assumed that both these groups are normally distributed on an underlying continuous scale, it is possible to establish the distance between the threshold and the mean for each group in terms of a number of standard deviations. This is done simply by applying the proportion of affected individuals in each group to tables of the normal distribution, like that shown in Table 2.3. Fig. 2.2 c and d show the normal curves corresponding to the two groups of mice, assuming that they both have the same standard deviation, with the means of both distributions expressed relative to the threshold on the standard deviation scale (for example, $\bar{X} = -1.28\sigma$, Fig. 2.2d). The difference between groups can now be given by the distance between means on the underlying continuous scale rather than simply by a difference in the proportion affected.

A simple comparison between groups can be made in terms of means arrived at in this way, but such a comparison suffers from the possible unjustified assumption that the standard deviations of the groups being compared are the same. In order to compare the spread of different groups, and also to make a better comparison of means, the quasicontinuous variable must be capable of being scored in three categories rather than two: nonaffected, minimally affected, and moderately to maximally affected. In such a situation there are therefore two

thresholds. Threshold 1 separates nonaffected from minimally affected, and threshold 2 separates minimally affected from moderately to maximally affected. The standard deviations of the groups can then be expressed in terms of what is assumed to be a constant interval between the two thresholds. Fig. 2.3 illustrates how comparisons of this sort can be made. Applying proportions (q) of each group to tables of the normal distribution, q_{a1} gives x_{a1}, the distance of threshold 1 from the mean of distribution a; and q_{a2} gives x_{a2}, the distance of threshold 2 from the mean of distribution a. Both these distances are in terms of σ_a, the standard deviation of distribution a. A similar procedure is adopted for distribution b. Thus the interval between the two thresholds, i, is given by $i = (x_{a2}-x_{a1})\sigma_a = (x_{b2}-x_{b1})\sigma_b$. If, for the sake of simplicity, i is defined as unity, or one threshold unit, the standard deviations of the two groups are: $\sigma_a = 1/(x_{a2}-x_{a1})$ threshold units, and $\sigma_b = 1/(x_{b2}-x_{b1})$ threshold units. It follows that the mean of distribution a, $\bar{X}_a$, is given by $\bar{X}_a = -x_{a1}\sigma_a$ threshold units from threshold 1, and the mean of distribution b, $\bar{X}_b$, is given by $\bar{X}_b = -x_{b1}\sigma_b$ threshold units from threshold 1.

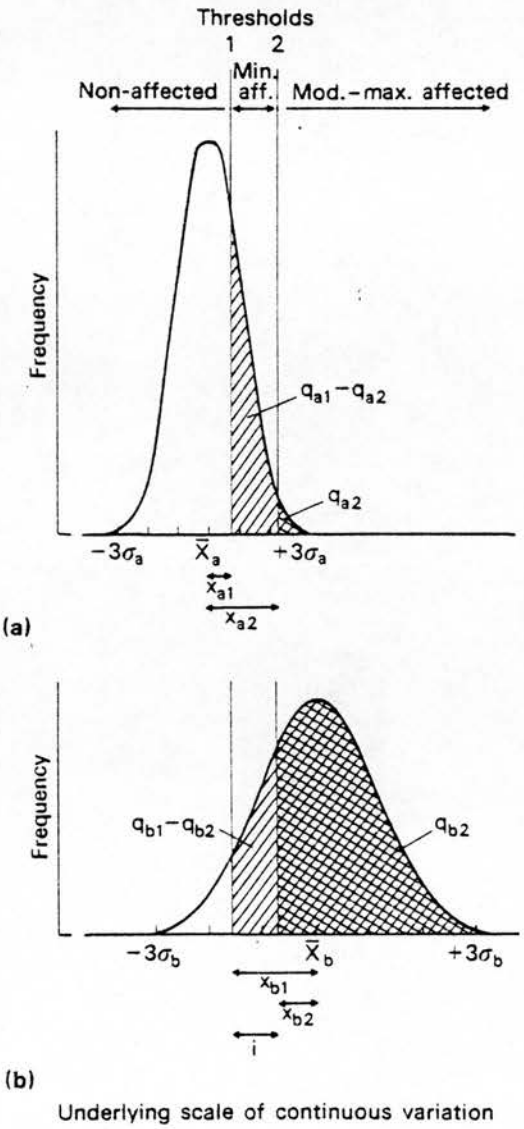


Fig. 2.3. Quasicontinuous variation with two thresholds. A value for x can be derived from tables given the appropriate proportion, q; and standard deviations and means can be compared in terms of the threshold interval, i.

Defining the position and spread of groups on the underlying continuous scale is perhaps one step closer to understanding the biological basis of quasicontinuous variation than simple consideration of the proportions of groups falling into two or three different classes.

DISCONTINUOUS VARIATION

An example of the last type of variation, discontinuous variation, is found in the dentition of the rice rat, a rodent native to central America. In a particular strain of these animals the molars, instead of remaining separate, frequently become fused together during development. Fusion may affect the molars of one, two, three or all four quadrants (each quadrant being the right or left side of the upper or lower jaw in each animal), or no quadrants at all. The number of quadrants affected is therefore always an integer. Table 2.4 shows the distribution of a large group of rice rats from the 'fused molar' strain over these five categories. Assuming that the number of quadrants affected is dependent on an individual's position on an underlying scale of continuous variation divided by four thresholds, and that the group of animals is normally distributed over this scale, reference to tables of the normal distribution can locate each threshold relative to the mean of the rice rat distribution in terms of this distribution's standard deviation. This is illustrated in Fig. 2.4. A point of interest to emerge is that the one-quadrant and three-quadrant categories, those categories that necessarily contain asymmetrical individuals, each occupies a range on the scale

Table 2.4. The distribution of a group of rice rats from the 'fused molar' strain according to the number of quadrants affected by molar fusion.

	Quadrants affected					
	0	1	2	3	4	Total
Number of animals	113	101	453	304	1209	2180
Percentage	5	5	21	14	55	100

about half the size of the two-quadrant interval. The two-quadrant category contains predominantly individuals with either two upper or two lower quadrants affected, in other words symmetrically affected animals. The level on the underlying scale therefore has to change twice as much to move from one end of this symmetrical category to the other as it does to move across either the one-quadrant or the three-quadrant interval, implying greater developmental stability of the symmetrical condition.

DIFFERENCES OF POSITION AND SPREAD

The general features of biological variation described in previous pages have been discovered by observation and experiment in a very large number of situations. It is appropriate now to consider how to interpret a particular observed difference, the basis of which is not yet understood. The approaches to this problem given here are largely concerned with differences between individuals or groups of individuals for continuous variables, but similar principles can be applied to quasicontinuous and discontinuous variables and to differences operating within individuals. A later section deals with the analysis of differences between groups for discrete variables.

Sampling

It is usually quite impractical to study an entire population or every example of a particular biological situation. Investigators must therefore be content to take samples; but how representative of an entire population, or every situation of a particular type, is a sample likely to be?

Suppose that the group of teeth in Fig. 2.1 b represents a complete population from which samples are drawn at random. The distributions of five random samples of ten teeth and five random samples of fifty teeth from this 'popula-

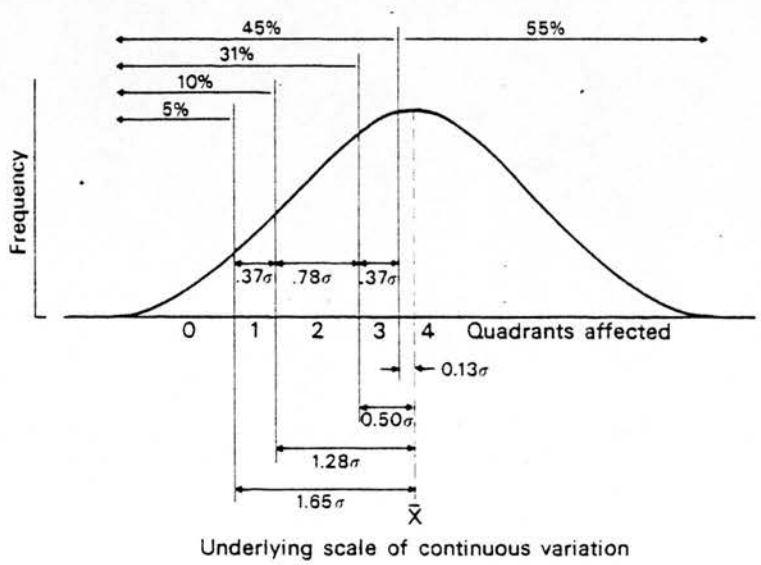


Fig. 2.4. The assumed normal distribution of the group of rice rats from Table 2.4 on an underlying scale of continuous variation divided by four thresholds. The distance of each threshold from the mean, $\bar{X}$, is shown in terms of σ, the standard deviation, as are the sizes of the intervals between adjacent thresholds.

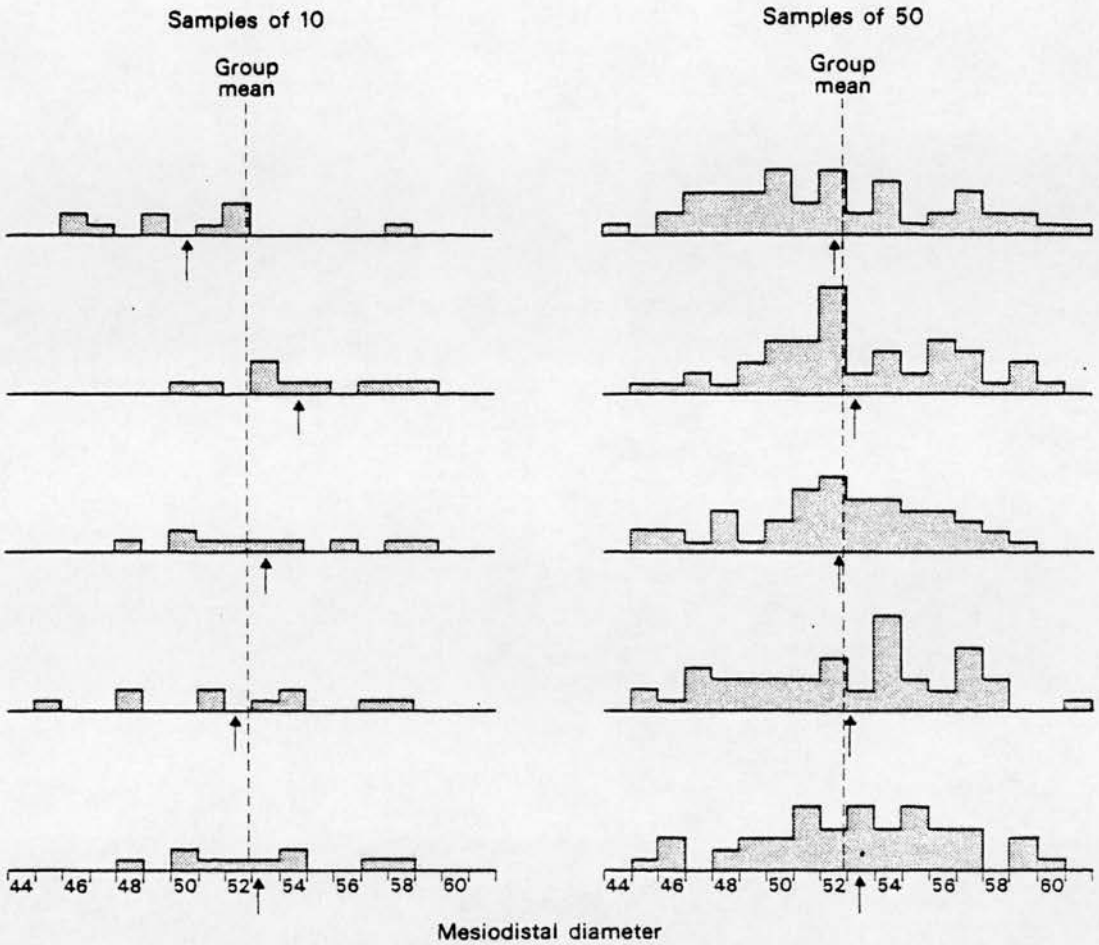


Fig. 2.5. Samples of ten and fifty taken at random from the group of teeth shown in Fig. 2.1b. The mean of the group from which the samples were drawn is shown by the broken vertical lines, and the samples means are shown by arrows. The average spread of samples of ten is 12.0 units, and the average spread of samples of fifty is 16.4 units of the scale. 1 unit = 10 μm.

tion' are shown in Fig. 2.5. The most obvious finding is that the means of the large samples agree much more closely with the mean of the whole group than the means of the small samples. In other words, the standard deviation of sample means from the true mean of the group is smaller for larger samples. The standard deviation of sample means from the true mean of a population is known as the standard error of the mean, and is symbolized by $\sigma_{\bar{x}}$. If the population is normally distributed (or nearly so), the means of samples of a given size are also normally distributed. The standard error is therefore exactly the same kind of statistic as the standard deviation. For instance, if in the present example each sample were composed of only one tooth, the standard error would be equal to the standard deviation, σ, of teeth in the group as a whole. It can be shown that the standard error decreases from this maximum value with increasing values of $\sqrt{n}$, the square root of sample size. The standard error is therefore given by $\sigma_{\bar{x}} = \sigma/\sqrt{n}$.

The standard error is used in attaching a level of reliability to the mean of a sample as an indicator of the true mean of the population from which the sample was drawn. The larger the standard error, the more unreliable the sample mean. Reliability is expressed by giving a range of values together with an associated level of confidence that the true mean of the population lies within the range. This can be accomplished using Table 2.3, where the values for standard deviations also apply to standard errors (because

the standard error is itself a standard deviation, that of sample means). The implication of Table 2.3 in this context is that when a large number of samples is taken from a given population their means fall, for example, within the range $-\sigma_{\bar{x}}$ to $+\sigma_{\bar{x}}$ (that is, one standard error on each side of the true mean) 68.2% of the time. The converse must also apply, namely that the true mean lies somewhere within one standard error on each side of the sample mean, also in about sixty-eight samples out of every 100. A greater level of confidence can be given if the range is widened. The true mean lies within two standard errors on each side of the sample mean in about ninety-five samples out of every 100, or within three standard errors in very nearly all samples (Table 2.3). Thus, once a value for $\sigma_{\bar{x}}$ has been calculated from a sample, the reliability of the sample mean can be expressed in terms of ranges on the scale of measurement.

A further point to notice in Fig. 2.5 is that the average spread of each sample on the scale, the distance on the scale between the smallest and largest items in the sample, is smaller for small samples than for large samples. Thus, whereas the mean of a sample is an unbiased estimate of the true mean of the population from which it comes, the standard deviation calculated from a sample is biased, always tending to underestimate the true spread of the population. This underestimation becomes more marked as sample size is reduced.

It has already been shown that the standard deviation of a complete group is given by $\sigma = \sqrt{\Sigma x^2/n}$, but it is apparent now that if this formula is applied to a sample it will underestimate the true standard deviation of the population from which the sample was drawn. What is needed is some sort of correction for this bias that will have a larger effect in smaller samples. It can be shown that a good correction for bias is achieved by substituting $n-1$ for n, so that the corrected, unbiased estimate of the standard deviation of a population, made from a sample of size n, is given by $\sigma = \sqrt{\Sigma x^2/(n-1)}$. The larger the sample the less effect the correction will have.

The quantity $n-1$ is known as the number of degrees of freedom. The reason for this may be illustrated as follows. Suppose that the mean of ten measurements is 100. Values for nine of these can be freely assigned; that is, each of nine measurements could theoretically take on any value from a very large negative number to a very large positive number, but the tenth can only have the value that makes the mean of all the measurements equal to 100. In a sample of n items there are therefore $n-1$ degrees of freedom.

Variance

As already mentioned, the square of the standard deviation, σ^2, is known as the variance. It is also sometimes symbolized by V. (The symbols σ^2 and V are normally used in slightly different contexts, but this distinction is not made here). The unbiased estimate of the variance of a population, made from a sample of size n, is therefore given by $V = \sigma^2 = \Sigma x^2/(n-1)$. The variance, like the standard deviation, is an indication of spread on the scale, but the variance has particular properties that make it useful for certain kinds of statistical analysis. The most important of these properties is the additive nature of the variance. This is best explained using an example. The diameter of the crown of a tooth is dependent both on the width of the dentine core and on the combined thickness of enamel on opposite faces of the crown. The width of the dentine core and enamel thickness vary from one individual to another, so there is a variance for dentine width, say V_d, and a variance for enamel thickness, say V_e. Assuming V_d and V_e to be independent of each other, the variance of crown diameter, say V_c, which is equivalent to the variance of the sum of the two measurements, symbolized by V_{d+e}, is simply the sum of the two variances. Thus $V_c = V_{d+e} = V_d + V_e$. What may appear surprising is that the variance of the difference between dentine width and enamel thickness, symbolized by V_{d-e}, is the same, that is, $V_{d-e} = V_d + V_e$. The reason for this is that two sources of variation contribute to the difference between dentine width and enamel thickness, as well as to their sum. These properties of variance are referred to later.

Differences between samples

There are two common kinds of statistical tests. First, there are tests for deciding whether a particular sample is likely to have been produced by the same general set of circumstances as that prevailing in a known situation; in other words, whether the sample is likely to have come from a known 'population'. Second, there are tests for deciding whether two samples are likely to be results of common influences, even though the nature of these influences may not be understood; or, put another way, whether the two samples are likely to have come from a single

'population'. In the sense used here and in the rest of this chapter, 'population' refers to groups not only of living individuals but also, for example, of fossil jaw bones or red blood cells.

THE NULL HYPOTHESIS

The samples in Fig. 2.5 obviously differ from each other. However, since they have all been made by random choice of individual teeth from the same group of third molars, the differences between them are due to nothing more than random sampling variation. Another way of saying this is that the differences are due to chance alone. The procedure for interpreting a difference of unknown origin, for example a difference between two samples, is to start with the hypothesis that the two samples are in fact derived from the same population. This is known as the null hypothesis, the hypothesis that there is no 'real' difference between the samples. A statistical test is then made, and if the result shows that the difference can reasonably be accounted for by chance alone the null hypothesis is accepted; that is, it is concluded that the samples come from the same source. Alternatively, if the test indicates that the observed difference is unlikely to have arisen by chance alone the null hypothesis is rejected and the difference between samples is said to be statistically significant. When this occurs, it is reasonable to accept that the difference is due, at least in part, to a real difference of circumstances that produced the different samples. Some simple statistical tests will now be described.

DIFFERENCES INVOLVING MEANS

Between the mean of a single sample and a known value

Suppose that an investigator wants to know whether the mean, $\bar{X}$, of a sample is significantly different from a value A, the known mean of a population. In other words, in terms of its position on the scale, is the sample likely to have come from this population? To answer this question the observed 'error', the difference between the sample mean and the mean of the known population, is compared with the standard error associated with the sample mean. (It will be recalled that the standard error is the difference from the true mean of the source population that is exceeded by sample means 31.8% of the time—Table 2.3). Under the null hypothesis, the population from which the sample was drawn is taken to be the known population itself. The investigator can therefore decide whether the observed error, in terms of the standard error assumed to apply to the known population, is small enough to be accounted for by chance alone. If so, he must conclude that there are no grounds for suspecting that the given sample has come from any other than the known population.

The ratio of the observed error to the standard error is distributed in the same way as a statistic known as Student's t; 'Student' being the pseudonym of W. S. Gosset, who introduced the statistic in 1908. Thus, $t = |\bar{X} - A| / \sigma_{\bar{X}}$, where $|\bar{X} - A|$ stands for the absolute value of the difference between the sample mean and the mean of the known population, a positive value no matter whether $\bar{X}$ is larger or smaller than A. The larger the difference between the sample mean and the mean of the known population the larger the value of t. Tables have been drawn up to show values of t for different degrees of freedom and different levels of confidence. The t values for, say, the '5% level' (the 5% level of significance, or the 95% confidence limits) are those which would be exceeded by chance alone in only 5% of samples. In other words, the mean of one in every twenty samples taken from the same population, when tested against the population mean, is expected to give a t value greater than that shown for the 5% level and the appropriate number of degrees of freedom. Yet another way of saying this is that the probability of this difference occurring by chance alone is less than 5%, symbolized by $P < 0.05$. Samples giving probabilities of less than 5% are generally regarded as being significantly different from the population against which they are being tested, and are therefore accepted as coming from a different source. Table 2.5 lists values of t for the 5% and 1% levels of significance (the 95% and 99% confidence limits) and for a few selected degrees of freedom.

Between the means of two samples

Suppose now that an investigator is comparing two samples, one with the other, and wants to know whether the difference between their means can be attributed to chance alone. In other words, is it likely that the two samples have been drawn from the same source? It is relevant here to have in mind the kind of distribution that results from plotting the difference between two sample means for a large number of pairs of samples drawn at random from the same population, a distribution that expresses the relative frequency with which differences of various size

Table 2.5. Values of t for two levels of significance and a few selected degrees of freedom.

Degrees of freedom	Probability of a larger t value occurring by chance alone: 5% (0.05)	1% (0.01)
1	t = 12.71	t = 63.66
2	4.30	9.93
3	3.18	5.84
4	2.78	4.60
5	2.57	4.03
6	2.45	3.71
7	2.37	3.50
8	2.31	3.36
9	2.26	3.25
10	2.23	3.17
20	2.09	2.85
50	2.01	2.68
100	1.98	2.63

occur. If the population is normally distributed (or nearly so), so that the distribution of means of samples of a given size is also normal, the difference between sample means is normally distributed too. Consequently, a standard deviation of the difference between sample means, known as the standard error of the difference, can be calculated. The investigator can therefore assess the difference between his two samples in a way similar to that used for testing a single sample against a known population; by comparing the observed difference between sample means with the standard error of the difference. One standard error (of the difference) is the difference that would be exceeded in about thirty-two pairs of samples out of every 100 drawn from the same population (Table 2.3).

The standard error of the difference between sample means is calculated as follows. The variance of sample means is the square of the standard deviation of sample means (the square of the standard error), that is, $\sigma_{\bar{X}}^2$. Because of a property of variances already referred to, the variance of the difference between two sample means, symbolized by σ_d^2, is given by the sum of the variances of sample means. Thus, $\sigma_d^2 = \sigma_{\bar{X}1}^2 + \sigma_{\bar{X}2}^2$, where the variances $\sigma_{\bar{X}1}^2$ and $\sigma_{\bar{X}2}^2$ are the squares of the two standard errors, $\sigma_{\bar{X}1}$ and $\sigma_{\bar{X}2}$, calculated for the two samples being compared. The square root of the variance of the difference, σ_d, is the standard deviation of the difference between sample means, and this is the standard error of the difference. The ratio of the observed error (the difference between sample means) to the standard error (of the difference) is again distributed as Student's t. Thus, $t = |\bar{X}1 - \bar{X}2| / \sigma_d$. The appropriate number of degrees of freedom is $n_1 + n_2 - 2$, where n_1 and n_2 are the sizes of the two samples being compared.

DIFFERENCES BETWEEN VARIANCES

A comparable test can be applied to variances. What is required is simply the ratio of the two variances being compared. The larger variance is always divided by the smaller so that the variance ratio, symbolized by F, is always greater than unity. Tables of F values are available for different levels of significance and various degrees of freedom. Two different degrees of freedom are required for each comparison, $n_1 - 1$ and $n_2 - 1$, where n_1 and n_2 are the sizes of the groups being compared. Table 2.6 shows values of F for the 5% and 1% levels and for a few selected degrees of freedom. The F values for, say, the 5% level are those which would be exceeded by chance alone in only one out of every twenty pairs of samples drawn from the same population.

As an example, consider again the two groups of mouse lower third molars illustrated in Fig. 2.1. For the two groups n = 139 and 141, and σ^2 = 4.5 and 12.3. The variance ratio is given by $F_{139}^{141} = 12.3/4.5 = 2.73$. Reference to Table 2.6 shows that this ratio exceeds the value given for F_{100}^{100} at the 1% level (that is, the value given for degrees of freedom closest to but less than 139 and 141), so the probability of this difference occurring by chance alone must be less than 1%. There is therefore good reason to believe that the variances of the two groups have been produced by sets of biological circumstances that are fundamentally different.

Problems of scale

Table 2.7 shows the mean and standard deviation of upper first molar width in one human and one mouse sample. Measured in millimetres, the standard deviation for the mouse molars is less than one tenth that for the human molars. Does this mean that upper first molars vary much less from one mouse to another than they do from one person to the next? In purely numerical terms this is certainly so. However, a given difference between two teeth, say a difference of 0.2 mm, has a much greater biological implication in mice than it does in man. This is a difference of four standard deviations in the quoted mouse sample, one that would be exceeded only

Table 2.6. Values of F for two levels of significance and a few selected degrees of freedom. Values in the upper row for each number of degrees of freedom correspond to a probability of 5%, and those in the lower rows to a probability of 1%; that is, values of F larger than those shown occur by chance alone only 5% and 1% of the time.

Degrees of freedom for smaller variance	Degrees of freedom for larger variance									
	5	6	7	8	9	10	20	50	100	200
5	5.05	4.95	4.88	4.82	4.78	4.74	4.56	4.44	4.40	4.38
	10.97	10.67	10.45	10.27	10.15	10.05	9.55	9.24	9.13	9.07
6	4.39	4.28	4.21	4.15	4.10	4.06	3.87	3.75	3.71	3.69
	8.75	8.47	8.26	8.10	7.98	7.87	7.39	7.09	6.99	6.94
7	3.97	3.87	3.79	3.73	3.68	3.63	3.44	3.32	3.28	3.25
	7.46	7.19	7.00	6.84	6.71	6.62	6.15	5.85	5.75	5.70
8	3.69	3.58	3.50	3.44	3.39	3.34	3.15	3.03	2.98	2.96
	6.63	6.37	6.19	6.03	5.91	5.82	5.36	5.06	4.96	4.91
9	3.48	3.37	3.29	3.23	3.18	3.13	2.93	2.80	2.76	2.73
	6.06	5.80	5.62	5.47	5.35	5.26	4.80	4.51	4.41	4.36
10	3.33	3.22	3.14	3.07	3.02	2.97	2.77	2.64	2.59	2.56
	5.64	5.39	5.21	5.06	4.95	4.85	4.41	4.12	4.01	3.96
20	2.71	2.60	2.52	2.45	2.40	2.35	2.12	1.96	1.90	1.87
	4.10	3.87	3.71	3.56	3.45	3.37	2.94	2.63	2.53	2.47
50	2.40	2.29	2.20	2.13	2.07	2.02	1.78	1.60	1.52	1.48
	3.41	3.18	3.02	2.88	2.78	2.70	2.26	1.94	1.82	1.76
100	2.30	2.19	2.10	2.03	1.97	1.92	1.68	1.48	1.39	1.34
	3.20	2.99	2.82	2.69	2.59	2.51	2.06	1.73	1.59	1.51
200	2.26	2.14	2.05	1.98	1.92	1.87	1.62	1.42	1.32	1.26
	3.11	2.90	2.73	2.60	2.50	2.41	1.97	1.62	1.48	1.39

rarely if pairs of teeth were drawn at random many times from this group of mice; whereas it represents less than one third of a standard deviation in the human sample, a difference that would be exceeded frequently if pairs of teeth were drawn at random many times from this human group. Any biological deductions made from the spread of a distribution on the original scale of measurement must therefore take into account the position of the distribution on the scale. The usual way in which this is done is to indicate the level of variability by the coefficient of variation, the standard deviation in terms of (divided by) the mean. When expressed as a percentage the coefficient of variation is therefore given by $CV\% = 100\sigma/\bar{X}$. Table 2.7 shows that the coefficients of variation of the human and mouse groups, although not the same, are very much more alike than the standard deviations. For their size, mouse molars therefore vary among themselves about as much as human molars.

Table 2.7. The mean ($\bar{X}$), standard deviation (σ) and coefficient of variation (CV%) for the width (buccolingual diameter) of upper first molars in one large human sample and one large mouse sample. Measurements for both samples were made in millimetres.

Sample	$\bar{X}$	σ	CV%
Human	12.05	0.71	5.9
Mouse	1.06	0.05	4.7

Sometimes comparable scale effects operate within a single group spread over a wide range of the scale, resulting in an asymmetrical or skewed distribution with, for positive measurements, the highest point of the distribution curve shifted to the left of centre. Logarithmic transformation of the measurements may be effective in eliminating skewness to produce a more or less normal distribution, allowing statistical procedures based on the normal curve to be applied. Other kinds of transformation may be appropriate under other circumstances.

VARIATION FROM MORE THAN ONE SOURCE OR WITH MORE THAN ONE EFFECT

A single variable may be subject to more than one influence. As already mentioned, tooth crown size is dependent both on the size of the

dentine core and on enamel thickness. However, restricting considerations to the outside of the crown alone provides no information about the dentine and enamel of which it is composed. Demonstration of a significant difference, for example between two groups for tooth size, therefore provides no information about the sources of variation that are responsible for the difference, or the relative sizes of their contributions. Conversely, two or more variables may be affected by a single common source of variation. The size of the dentine core and the thickness of enamel are probably dependent to some extent on common nutritional and metabolic factors, but studying the dentine and enamel separately will not disclose such an association between them. Statistical methods are therefore available for assessing the relative importance of different sources of variation, and for analyzing the way in which two or more characters vary together.

Before going into this in a little more detail, it should be mentioned that if dentine width and enamel thickness are found to have a common source of variation, the equation $V_c = V_d + V_e$, referred to earlier, does not apply (unless the common source can be shown to have a relatively small effect). This is because the commom source variance contributes to both V_d and V_e, causing it to be represented twice instead of only once in the right hand side of the equation. The size of V_c therefore becomes progressively smaller than $V_d + V_e$ as the common source variance increases.

Components of variance

In many other situations too, the total observed variation can be partitioned into components associated with different sources of variability. For example, two examiners may be scoring dental caries in children of the same age group at a number of different schools. To check on the accuracy of the scoring procedure they may choose to score each child on two separate occasions. If all caries scores collected in this way were considered together there would be an overall variance of caries score, but differences between scores may have arisen for three different reasons. First, there may be a real difference between schools, perhaps due to a preponderance of different dietary habits. Second, there may be a difference between examiners, one examiner consistently recording higher scores than the other because of slightly different scoring criteria. Third, the caries score for each individual may not be exactly the same for the two occasions on which it was recorded, a difference due to imperfect repeatability.

The relative importance of different sources of variation in a situation of this kind can be assessed by what is known as analysis of variance. As already shown, the unbiased estimate of the variance of a population, made from a sample of size n, is given by $V = \sigma^2 = \Sigma x^2/(n-1)$. The quantity Σx^2, the sum of squared deviations from the mean, is often referred to as the sum of squares. The variance is therefore equal to the sum of squares divided by the number of degrees of freedom. Suppose that there are s samples each composed of n items, so for the sake of simplicity only two possible sources of variation are being considered, 'within sample' and 'between sample' variation. Under the null hypothesis there is no real difference between samples; in other words the assumption is that they have been drawn from the same population. Sums of squares and degrees of freedom are first computed for each sample separately. For each of the s samples, this gives a value for Σx^2 (the sum of squared deviations from their own sample mean) with $n-1$ degrees of freedom. The variance within samples, V_W, is the sum of all these within sample sums of squares, symbolized by $\Sigma\Sigma x^2$, divided by the sum of all their degrees of freedom, $s(n-1)$. Thus, $V_W = \Sigma\Sigma x^2/s(n-1)$. Assuming the null hypothesis to be correct, this observed within sample variance provides an estimate of σ^2, the true variance of the population from which the samples were drawn. It has already been shown that the variance of the means of samples drawn from the same population is given by $\sigma_{\bar{x}}^2 = \sigma^2/n$. Thus, if the null hypothesis is correct, the observed variance of sample means, $V_{\bar{x}}$, should not differ significantly from V_W/n, the expected variance of sample means if all samples come from the same population. This difference can be tested using the variance ratio $F = V_{\bar{x}} : V_W/n = nV_{\bar{x}}/V_W$, with $s-1$ degrees of freedom associated with the variance of sample means and $s(n-1)$ degrees of freedom associated with the within sample variance. If the F test shows that there is a significant difference between these variances it can be concluded likely that the samples have been drawn from more than one population with means that differ from each other.

The difference between the observed variance of sample means, $V_{\bar{x}}$, and that predicted by the null hypothesis, V_W/n, is the between sample component of variance, V_B. Thus, $V_B = V_{\bar{x}} - (V_W/n)$. Once the null hypothesis has been rejected it is concluded that individuals are dif-

ferent partly because of within sample variation and partly because of variation between samples. The relative size of these two components indicates the relative importance of within and between sample sources of variation. Other forms of analysis of variance can be applied to more complex situations where several sources of variation are considered at the same time.

Regression and correlation

So far only a single variable, symbolized by X, has been considered. Suppose now that there is some reason for studying two variables, X and Y, at the same time. For example, does an individual with a large upper jaw also have a large lower jaw; and likewise, does a small upper jaw go with a small lower jaw? In more general terms, do the sizes of upper and lower jaws tend to vary together from one individual to another?

Consider how the two variables X and Y might be related. The simplest (and luckily the most frequent) kind of relationship can be represented graphically by a straight line. It could be that Y is simply proportional to X, so that Y is equal to X multiplied by some unknown constant, say b. In such a case $Y = bX$. For example, if $b = 1$ the value of Y is always equal to the value of X, if $b = 2$ the value of Y is always twice that of X, and if $b = \frac{1}{2}$ the value of Y is always half that of X. The value of b is known as the slope of the line (Fig. 2.6a). If the relationship between X and Y is always exact, only one pair of corresponding X and Y values is required to find b (from the equation $b = Y/X$). However, this is extremely unlikely. The general trend of the association between X and Y may be described by a straight line, but the association is almost never perfect so that different X,Y pairs usually give different values of b. A series of X and Y values is therefore required to arrive at a slope for the line that is representative of the situation as a whole.

Furthermore, straight lines expressing the relationship between two variables do not always pass through the origin, the point corresponding to zero on both axes of the graph. The full equation for a straight line is given by $Y = bX + a$, where a is the point where the line intersects the Y axis (Fig. 2.6b). Values for both a and b are required to describe a straight line relationship fully. The question is how to arrive at values of a and b for a sample of X and Y measurement pairs. The values required are those specifying the 'best straight line' for the particular set of data. One thing that can be seen intuitively is that this line must pass through the point $(\bar{X}\bar{Y})$, corresponding to the means of X and Y values, but a second point through which the line passes must be known before the line can be drawn.

Returning for a moment to the case of a single variable, X, it can be shown that the sum of squared deviations of individual measurements from any point selected on the scale is a minimum when the selected point is at the mean, $\bar{X}$. If deviations are taken from any other point on the scale their sum is larger than Σx^2. This principle of least squares is used to arrive at the best fitting straight line. The value of b, the regression coefficient, for the best straight line is known as the regression of Y on X and is given by $b = \Sigma xy/\Sigma x^2$, where $x = X - \bar{X}$ as before, and where $y = Y - \bar{Y}$. The quantity Σxy is the sum of products of the deviations x and y for each pair of X and Y measurements. Since the value of b can be calculated in this way, and

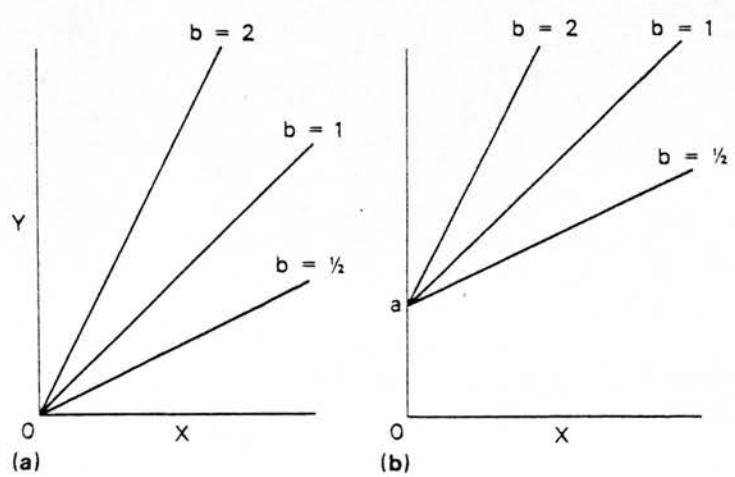


Fig. 2.6. Different straight line relationships between two variables, X and Y. The slope of the line is given by b.

since $\bar{X}$ and $\bar{Y}$ are known, the value of a can be found from the equation $a = \bar{Y} - b\bar{X}$. There are now two points through which the best straight line is known to pass, $(\bar{X},\bar{Y})$ and $(X=O, Y=a)$, so the line can be drawn. The line produced in this way is that from which the sum of squared deviations of Y values is at its lowest.

The null hypothesis here is that Y is unrelated to X. In other words, variation in Y has nothing to do with variation in X, and the true value of the regression coefficient, b, is zero; but how is an investigator to know whether the value of b he has calculated reflects a real relationship between X and Y or whether it occurred by chance alone in a situation where no real relationship existed? The level of confidence in the estimated value being significantly different from zero is taken from tables of F values, the same tables that are used to test variance ratios. The value of F is given by

$$F = \frac{b\Sigma xy(n-2)}{\Sigma y^2 - b\Sigma xy}$$

where n is the number of pairs of X and Y measurements. One degree of freedom is associated with the numerator and $n-2$ degrees of freedom with the denominator. The standard error of the regression coefficient, given by $b/\sqrt{F}$, can be used to test for a difference between regression coefficients calculated for two samples.

The regression coefficient indicates the direction (in graphical terms) of the association between X and Y rather than its strength. The most usual way of expressing the strength of an association is by means of the correlation coefficient. This is a quantity that always falls between -1 and $+1$. A value of zero means that there is no association; a value of $+1$ means that there is perfect association, with Y increasing as X increases; and a value of -1 means that there is perfect association but that Y decreases as X increases. Different correlation situations are illustrated in Fig. 2.7. The correlation coefficient, symbolized by r, is given by

$$r = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \Sigma y^2}}.$$

The level of confidence that a calculated value of r is significantly different from zero is taken from special tables drawn up for this purpose (Table 2.8). There are also ways of testing for a difference between correlation coefficients calculated from two samples of unknown origin, but these are too involved to be mentioned here.

There are three kinds of reason for a significant correlation coefficient, and the investigator must use whatever other facts are available to decide which one applies in any given situation. The first is that the association stems from a direct cause and effect relationship as, for instance, between physical exertion and heart rate. Second, the two variables may not be directly related but affected by a common influence. For example, human development can be divided into stages according to either the state of ossification of the skeleton or the degree of calcification of the forming teeth. The developmental stages defined by ossification are highly correlated with those based on dental calcification, but not because the skeleton influences the dentition directly, or vice versa. Both are affected by the general body changes associated with growth and development. Third, the association may be entirely spurious, with no causal relationship between the two variables, either direct, or indirect through the operation of a common influence. This spurious association is what the significance test is designed to reduce to an acceptable level of probability.

DIFFERENCES OF FREQUENCY

Populations or groups of individuals may be characterized by the frequencies of the different forms of discrete variables (see, for example, Table 2.1). There are two kinds of question that can be asked here, analogous to those for which the two kinds of t test are used. The first is, are the frequencies in a sample compatible with the null hypothesis that the sample has come from a

Table 2.8. Values of the correlation coefficient at the 5% and 1% levels of significance. For an observed correlation to be regarded as significantly different from zero it must be equal to or larger than the value shown for the chosen level of significance and the appropriate number of degrees of freedom. The table applies to negative as well as positive values.

Degrees of freedom	5% (0.05)	1% (0.01)
5	0.75	0.87
6	0.71	0.83
7	0.67	0.80
8	0.63	0.77
9	0.60	0.74
10	0.58	0.71
20	0.42	0.54
50	0.27	0.35
100	0.20	0.25
200	0.14	0.18

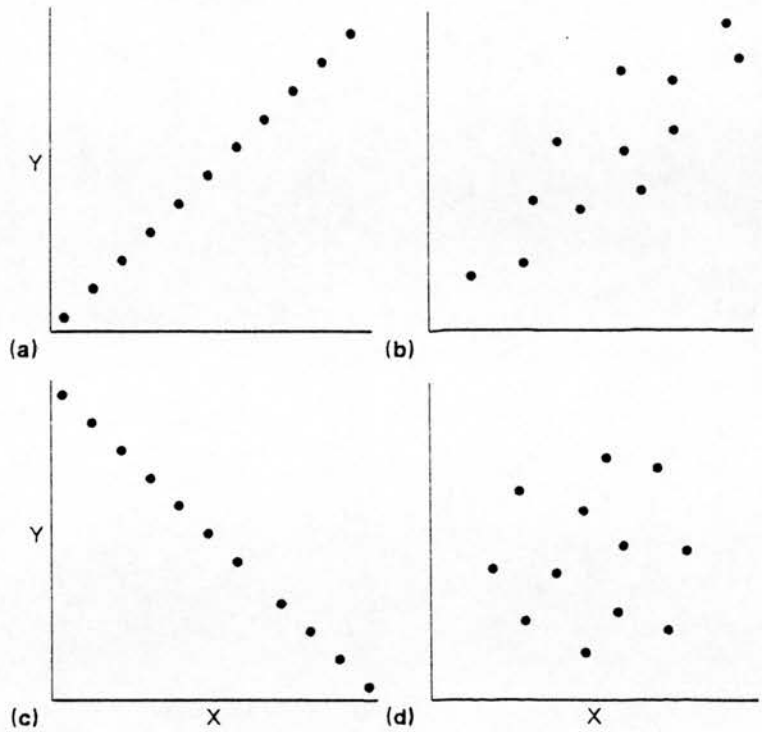


Fig. 2.7. 'Scatter diagrams' showing different kinds of association between two variables, X and Y. (a) shows perfect positive association, with r = 1 (and b = 1); (b) shows strong positive association, with r between 0.8 and 0.9 (and b = 1); (c) shows perfect negative association, with r = −1 (and b = −1); and (d) shows no association, with r = 0.

known population where the overall frequencies of the different forms have been established? For example, prior knowledge leads to the expectation of a 1:1 female to male ratio in human populations. The ratio of females to males in samples of human populations should therefore not differ significantly from a 1:1 ratio unless there are factors involved in making up the samples that tend to alter the ratio in one direction or the other. Suppose that in a class of dental students nineteen are females and thirty-one are males. Some of us may be all too ready to ascribe this difference to superior academic achievement of males, and others to poorer opportunity for females. (There are, of course, other possible reasons for a difference also.) However, before jumping to any rash conclusion, the data should be properly analyzed to see if there is any justification for accepting that the difference from a perfect 1:1 ratio is likely to be due to anything other than chance.

Table 2.9 shows how such an analysis is carried out. The observed numbers, O, are nineteen females and thirty-one males, with a total of fifty. The expected numbers, E, are based on the expected proportions (1:1) and the total (fifty), and so are twenty-five females and twenty-five males. The appropriate number of degrees of freedom is equivalent to the number of categories whose frequencies can be assigned arbitrarily. For example, in this case the frequency of females could take on any value, but

Table 2.9. The calculation of chi-square expressing the difference between observed and expected numbers. The symbol χ_1^2 means chi-square with one degree of freedom. The probability of this particular difference occurring by chance alone is greater than 5%, and the difference is therefore not regarded as statistically significant.

	Females	Males	Total
Observed (O)	19	31	50
Expected (E)	25	25	50
O–E	−6	6	
$(O-E)^2$	36	36	
$(O-E)^2/E$	1.44	1.44	2.88
$\chi_1^2 = \Sigma((O-E)^2/E) = 2.88$		P>5%	

once the female frequency is decided the frequency of males is fixed by the total. In these two categories there is therefore only one degree of freedom. (More than two categories are allowed in such a test, and the number of degrees of freedom is always one less than the number of categories.) The sum of the squared differences between observed and expected numbers, each squared difference expressed relative to the expected number for its own category, is given the symbol χ^2 (chi-square). Thus, chi-square is given by $\chi^2 = \Sigma((O-E)^2/E)$. Tables of chi-square values are available for different degrees of freedom and different levels of significance. The values of χ^2 for, say, the 5% level (the 95% confidence limits) are those which would be exceeded by chance alone in one out of every twenty samples drawn from the known population. Samples giving larger values are therefore regarded as significantly different from the expected proportions against which they are being tested, and are accepted as coming from a different source. Values of χ^2 for the 5% and 1% levels, and for a few selected degrees of freedom, are shown in Table 2.10. It can be seen that the value calculated from the example given in Table 2.9 would be exceeded by chance alone more often than 5% of the time. The difference between the sex ratio in the class of students and the expected 1:1 ratio is therefore not statistically significant, and there is no reason to suppose, on statistical grounds, that anything other than chance is responsible for the difference.

The second question is, given the frequencies of the different forms of a discrete variable in two samples, is it likely that the two samples have come from the same source? For example, suppose that a new treatment for a particular disease has been developed, and everyone is anxious to know whether it is better than the old treatment. Patients are assigned at random to the two treatment groups and the results of the treatment, in terms of the numbers of patients cured and uncured, are set up in the form of a contingency table, as shown in Table 2.11. The contingency table in Table 2.11 is a 'two-by-two' table, that is, it has two rows and two columns, but more than two rows and two columns are allowed. For instance, if three treatments were being tested there would be three rows, and if some of those individuals left uncured suffered a toxic reaction to treatment a third column could be added. The number of degrees of freedom in a contingency table is given by $(R-1)(C-1)$, where R is the number of rows and C is the number of columns. The numbers of patients in Table 2.11 have been represented by symbols. Using these sympols, chi-square is given by

$$\chi_1^2 = \frac{n(ad-bc)^2}{(a+b)\ (c+d)\ (a+c)\ (b+d)}$$

Values that exceed those given for the 5% level are generally taken to indicate that the two samples have been drawn from different sources; in other words, in this case, the treatments had different effects.

Table 2.10. Values of χ^2 for two levels of significance and a few selected degrees of freedom.

Degrees of freedom	Probability of a larger χ^2 value occurring by chance alone: 5% (0.05)	1% (0.01)
1	$\chi^2 = 3.84$	$\chi^2 = 6.63$
2	5.99	9.21
3	7.81	11.34
4	9.49	13.28
5	11.07	15.09

Table 2.11. A 'two-by-two' contingency table for calculating chi-square that expresses the difference between two samples. The symbols a, b, c and d stand for different numbers of patients.

	Patients cured	Patients not cured	Total
Old treatemt	a	b	a+b
New treatment	c	d	c+d
Total	a+c	b+d	a+b+c+d=n

$$\chi_1^2 = \frac{n(ad-bc)^2}{(a+b)\ (c+d)\ (a+c)\ (b+d)}.$$

CONCLUDING REMARKS

Whenever anything is measured numerically, some procedure is required for giving meaning to the numbers obtained. Statistical tests have been devised for this purpose. Statistics is concerned with probabilities that are invariably greater than zero (complete impossibility) and less than unity or 100% (absolute certainty). Statistical tests can therefore neither prove nor disprove anything absolutely. They can only indicate the relative likelihoods of alternative explanations. It is up to the

investigator to decide what level of probability he will regard as providing acceptable evidence for or against a particular hypothesis.

There are two problems for the user of statistical procedures. The first is the choice of an appropriate test for the situation in hand, and the second is the interpretation of the results of the test. The tests dealt with in previous pages are among the simplest and most commonly used in biology, and it is hoped that the explanations given are sufficient to provide the reader with some insight into a few relevant fundamentals of statistics. There are, however, many more tests in use, the application and interpretation of which may be difficult for anyone other than a professional statistician. This should not deter the biologist from using more sophisticated tests, since professional statisticians can easily be consulted when all but the simplest of procedures are being contemplated.

Finally, it may encourage the critical faculties of the reader to ponder on the following. Every year in the research literature thousands of results significant at the 5% level are quoted and taken as evidence of real differences. Five out of every hundred of these are suggesting a difference where no real biological difference exists. Which results are the misleading ones?

CHAPTER 2
Genetics

Genetics, in the classical sense, is concerned with the contribution to biological variation made by inherited differences between individuals. On a broader scale it is also concerned with differences between populations, as well as the changes that occur in the genetic characteristics of the same population from one generation to the next. At a finer level, genetics includes the study of how gene activity is controlled within individuals to produce differences of structure and physiology between various parts of the body. Some of these aspects are dealt with in Book 1. The aim of this chapter is to give a general overall view of how inherited differences, which are fundamentally biochemical, contribute to the variation observed between individuals and populations.

GENES, CHROMOSOMES AND GENETIC VARIATION

Genes are determinants of hereditary characteristics. Each gene is an item of stored information that either specifies the structure of a particular substance or can be used to control the activity of other genes. Genes also have the capacity for accurate self replication. The information contained in them can therefore be passed unaltered from one cell generation to the next, and from a parent to its offspring. It is important to realize that the existence of genes can only be inferred from variation in the characters they produce.

Genes and chromosomes

The majority of known genes are located in the chromosomes. The earliest and most compelling evidence for this was the discovery that the transmission of most inherited characters from one generation to the next parallels the transmission of the chromosomes themselves. The remaining few hereditary characters do not behave in this way, and it is concluded that these are controlled by non-chromosomal genes. Non-chromosomal inheritance is not as well understood as chromosomal inheritance and is of little relevance in the present context. The following discussions are therefore concerned only with chromosomal genes.

Gene is a rather indefinite term and it is often better to be more specific. A locus is the site occupied by a gene. Loci are arranged linearly along each chromosome. An allele is one of a number of alternative forms that a gene may take and different forms of the same gene are said to be allelic. For example, the MN blood group locus in man may be occupied by either an 'M substance' producing allele or an 'N substance' producing allele. Such allelic differences provide the basis for the genetic component of variation of any character. Conversely, it is the possession of common alleles, derived from a common ancestor, that contributes to the resemblance between relatives.

In biochemical terms, an allele is a unique nucleotide sequence and its locus is the position of this sequence in the very much longer linear series of nucleotides of a chromosome's DNA. The difference between one allele and another at the same locus may be limited to only one nucleotide position in the sequence, or it may occur at more than one position.

The body is composed of two classes of cells: the somatic cells, which form the substance of the individual, and the germ cells or gametes, which are the individual's potential contribution to the next generation. The nucleus of each human somatic cell normally contains 23 pairs of chromosomes. One member of each pair is contributed by each parent so that each pair can be said to comprise a maternally derived and paternally derived chromosome. The two members of a pair are known as homologous chromosomes or homologues. One of the 23 pairs is a pair of sex chromosomes, and members of the other 22 pairs are called autosomes. Each gamete contains only one member of each chromosome pair, and its chromosome complement is known as haploid. At fertilization one gamete from each parent unite to

form a single-celled zygote that subsequently develops into a new individual. As the zygote receives one member of each chromosome pair from each parent it is able to start its development with the full somatic, or diploid, complement of chromosomes.

The two members of each pair of autosomal chromosomes are potentially identical in that the same loci are present in the same sequence on both. The only differences are allelic. Each pair of autosomes is normally completely different from each of the others so that there are only two homologous autosomal loci of each type in a diploid cell, one on each member of a chromosome pair. If both alleles at a given locus are identical (it is usual to use 'locus' to refer to a pair of homologous loci) the individual is known as a homozygote (homozygous at that locus for that allele), and if the alleles are different the individual is known as a heterozygote (heterozygous at that locus).

The sex chromosomes and X-linked genes

The sex chromosomes differ from the autosomes in that there are two quite different forms. One is the X-chromosome, which can be regarded as comparable to an autosome. Loci carried by the X-chromosome are said to be X-linked (or sex-linked) but are not necessarily directly concerned with sexual differentiation. The other is the much shorter Y-chromosome. All viable individuals possess at least one X-chromosome. The sex of an individual is dependent on whether the other member of the sex chromosome pair is another X-chromosome, associated with femaleness, or a Y-chromosome, which confers maleness. The Y-chromosome therefore carries genes essential for male sexual differentiation, but no Y-linked loci corresponding to those on the X-chromosome have been demonstrated. Females can thus be either homozygous or heterozygous for any X-linked gene, just as for an autosomal gene, but as males possess only one allele at each X-linked locus they can be neither. Males are consequently said to be hemizygous at all X-linked loci.

Over the past few years there has been increasing interest in why males, with only one copy of each X-linked gene, have similar characteristics to females, with a double dose of each X-linked gene. More specifically, heterozygotes for enzyme deficiencies controlled by autosomal genes (such as the deficiency of phenylalanine hydroxylase that causes phenylketonuria) generally have half the enzyme activity of normal homozygotes, whereas enzymes controlled by X-linked genes, such as glucose-6-phosphate dehydrogenase, usually show the same level of activity in normal males and females, despite the difference in X-linked gene dosage (Book 1). It is widely accepted that dosage compensation results from random inactivation of one or other of the X-chromosomes in each somatic cell of females at an early stage of embryonic development so that only one of the two alleles at homologous X-linked loci is able to function in any cell. This action is irreversible, and the same chromosome remains inactive in all descendants of any given cell. Therefore, in a female heterozygous at an X-linked locus, about half the somatic cells will have one allele active and the other half will have the other allele active. This means that X-linked heterozygotes are mosaics made up of patches of two different cell types. This mosaicism may or may not be detectable.

Normal and abnormal chromosome behaviour

During growth and regeneration somatic cells give rise to more somatic cells by mitosis. Mitosis involves the replication of each chromosome so that when a parent cell divides each of its two daughter cells can receive the full somatic complement of chromosomes. The gametes are derived from somatic cells by meiosis. At the reduction division of meiosis there is cell division without chromosome replication, and, as a result, only one member of each chromosome pair passes into each gamete. For each chromosome pair it is purely a matter of chance whether a given daughter cell receives the maternally derived or the paternally derived chromosome. This chance distribution of maternal and paternal chromosomes at meiosis is known as independent assortment.

Prior to independent assortment, the members of each chromosome pair come into close contact with each other and corresponding segments may be exchanged. Therefore, the chromosomes passed into the gametes are often reconstituted, the maternally derived chromosome of each pair now containing some segments of paternal origin, and the paternally derived chromosome some segments of maternal origin. This exchange can occur anywhere along the chromosome and is known as recombination. Each chromosome segment exchanged by recombination carries many loci. Thus alleles at neighbouring loci tend to be transmitted together, whereas those at a greater distance from each other are more likely to be separated by the recombination process. The frequency with which two loci are separated by

recombination can often be determined and is a measure of their physical proximity on the chromosome, known as their degree of linkage.

Occasionally something goes wrong with the assortment of chromosomes at meiosis. If members of a chromosome pair fail to become separated after recombination, one daughter cell will receive both members of the pair and the other daughter cell neither. This failure of separation is known as non-dysjunction and is responsible for producing gametes, and through them individuals, with abnormal chromosome complements. For example, if non-dysjunction of the small autosome numbered 21 occurs at the reduction division of female meiosis, one of the gametes produced will carry both of these autosomes instead of one, and the other will carry neither. If each of these cells is later fertilized by a normal sperm two different types of abnormal zygote will be formed. One will have the two autosomes 21 of maternal origin and an additional autosome 21 from the father; that is, three copies of chromosome 21 in all, a condition known as trisomy 21. The other will have only one copy of chromosome 21 (monosomy 21) which was contributed by the father. Monosomic individuals are not viable but trisomy 21, which produces Down's syndrome (mongolism), is one of the most frequently found chromosomal abnormalities. Non-dysjunction occurs sporadically and at a low frequency.

Rarely, a segment of chromosome of one pair may become attached to a member of another pair. This is known as translocation, and again chromosome 21 may be involved. Part of chromosome 21 may become attached to one of the larger autosomes so that, at the reduction division of meiosis, one of the two gametes formed receives the normal larger autosome and the other the larger autosome carrying the translocation. In addition, one of the gametes receives the normal untranslocated chromosome 21. If the normal chromosome 21 and the autosome carrying the translocation pass into the same gamete, fertilization will produce a zygote with a greater amount of chromosome 21 material than normal; two free chromosomes (one from each parent) and, in addition, the translocated portion. The result again is Down's syndrome. If the normal chromosome 21 and the autosome carrying the translocation pass into different gametes, each will have a more or less balanced haploid complement. Fertilization of the gamete containing the translocated chromosome produces a zygote with a balanced diploid complement, though such individuals will themselves produce abnormal gametes carrying both the translocation and a free chromosome 21 which, when fertilized, result in trisomy 21. Individuals carrying the translocation may therefore be unaffected but can produce more than one offspring with Down's syndrome.

The maintenance of genetic variation

Alleles are not absolutely stable. Although they are usually transmitted unaltered from one generation to the next, rare events occur that cause changes within them. These events are called mutations, and an allele that has undergone such a change is transmitted in its new, mutant form.

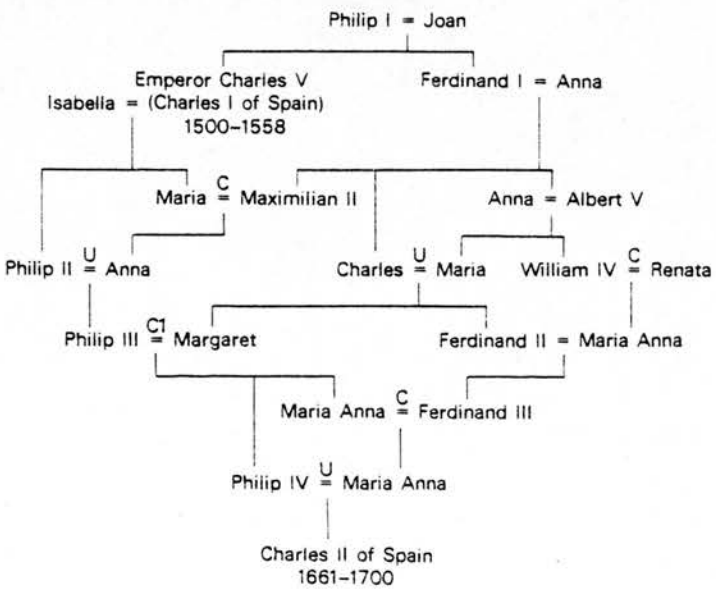


Fig. 2.1. Inbreeding among some of the Spanish Hapsburgs. In the interval between the emperor Charles V and his descendant Charles II of Spain there were three marriages between uncles and their nieces (U), three between first cousins (C) and one between first cousins once removed (C1).

Fig. 2.2. The emperor Charles V (left) by Christoph Amberger [Cat. No. 556—Detail—Staatliche Museen Preussischer Kulturbesitz, Gemäldegalerie, Berlin (West)]; and his descendant Charles II of Spain (right) by Juan de Miranda Carreño (Inv. No. 1714—Detail—Kunsthistorisches Museum, Gemäldegalerie, Vienna).

Mutation increases genetic variation by introducing new genetic material. Recombination and independent assortment allow new combinations of existing genetic material to arise, but the effectiveness of these processes in creating genetic diversity depends on the mating of genetically dissimilar individuals. Mating between close relatives, known as inbreeding, therefore results in a reduction of genetic variation. This is exploited in the laboratory to produce genetically homogeneous (or nearly so) animal strains by successive generations of brother × sister mating. In man, inbreeding is never so intense, but marriage between close relatives can lead to the persistence of inherited characteristics over several generations. The protruding lower jaw of the Hapsburgs, for instance, retained a high degree of expression on the Spanish side of the family for 200 years. Persistence of extreme expression was probably contributed to by inbreeding (Figs. 2.1 and 2.2).

GENOTYPE AND PHENOTYPE

The genetic constitution of an individual is known as his genotype. Genotype may refer to a specified locus or loci, or to all loci in general. An individual's phenotype is the final product of a combination of genetic and environmental influences. Phenotype may be used to refer to a specified character, or to all the observable properties of the individual taken together.

Genes and characters

Different types of character can be thought of as being different distances from the fundamental level of gene activity. The further a character is removed from this fundamental genetic level the greater the likelihood that its variation is dependent on allele differences at more than one locus, and also on environmental influences. Enzymes, for instance, are products of gene expression, and in most cases it has been shown that a single locus is responsible for the structure of a single enzyme. Characters dependent on the structure of a single enzyme therefore usually show variation that is directly related to allele substitution at a single locus. For example, the most common variety of albinism results from a lack of the enzyme tyrosinase, which is necessary for the formation of pigment (Book 1). At the tyrosinase locus normal individuals possess the allele responsible for producing a normal functional enzyme, whereas tyrosinase-negative albino individuals have at this locus other alleles that are unable to produce a functional enzyme.

Morphological characters, on the other hand, such as the almost infinite number of dimensions

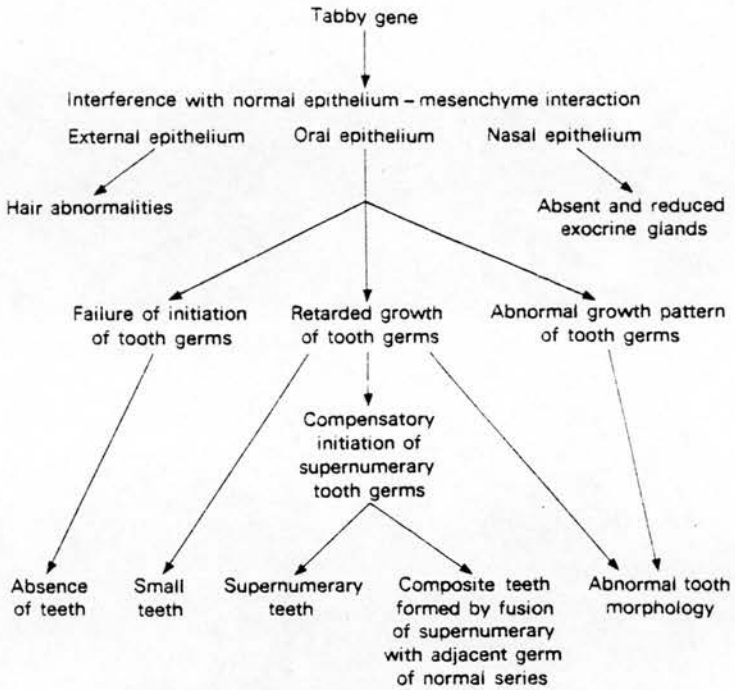


Fig. 2.3. Part of the hierarchy of developmental abnormalities caused by the mutant gene 'tabby' in the mouse.

that can be used to describe the shape of the face and jaws, are furthest removed from the fundamental genetic level and are the end results of a vast complexity of interacting developmental processes. Each gene is therefore likely to influence many morphological characters, and, since development has a basically hierarchical nature, the earlier a gene becomes active the more widespread and varied its effects are likely to be. Detectable single allele substitutions, although producing a unitary effect at the gene level, almost always result in syndromes of morphological abnormalities. When a gene is known to affect a number of different characters its action is said to be pleiotropic. For example, the most fundamental observed effect of the mutant gene 'tabby' in the mouse is an interference with the interaction between epithelium and its underlying mesenchyme that occurs during the formation of epithelial derivatives. As a consequence, these mutant mice have abnormalities of the hair, certain exocrine glands and the teeth. The teeth are generally reduced in size, of abnormal shape and sometimes absent altogether, but there may also be supernumerary teeth and composite teeth formed by fusion of two adjacent tooth germs (Fig. 2.3). The unitary action of the gene therefore has different manifestations in different developmental systems, and even within a system may have a variety of effects depending on the local conditions prevailing during development.

By contrast, the interaction that occurs between different tissues and different cell types at all stages of development allows allele substitutions at many different loci to have some effect on each morphological character. Consequently, variation in the majority of morphological characters studied cannot be shown to be due to allele substitutions at a single locus alone. Many genes and an environmental component are usually responsible. This has been demonstrated clearly for the absence of third molars in mice. Absence appears to be merely an extreme expression of small size, and is affected by many genes, diet and the lactational performance of the mother.

Modification of gene effects

Some genes produce the same phenotype under all known conditions, but the effects of others may be modified, either by the environment, by other genes, or both. When a mutant genotype produces an abnormal phenotype in some individuals but not in others it is said to have incomplete penetrance. If, among individuals who show the abnormal phenotype, the degree of abnormality varies, the phenotype is said to have variable expressivity.

GENOTYPE AND ENVIRONMENT

The interaction between genotype and the

external environment may fall into one of a number of categories, two of which are mentioned here. First, the same genotype may produce different phenotypes in different environments. In the United Kingdom, as in many other countries, the average height for any given age group has been increasing over several years. This does not appear to be due to a change in genetic composition of the population but rather to an improvement in nutritional status and medical care. Secondly, different genotypes may react differently to the same environmental change. A normal diet causes severe mental disability in individuals with phenylketonuria but, whereas in early diagnosis a low phenylalanine diet makes all the difference between disability and mental normality to phenylketonurics, it has no comparable effect on normal individuals.

In addition to the external environment there is the internal environment of the developing individual. At any stage of development it is reasonable to assume that the same genes are active on both sides of the body, yet bilaterally represented structures regularly fail to form as exact mirror images of each other. This is partly due to differences in the local environment around the developing structures on the two sides, differences which, as far as the dentition is concerned, can lead to asymmetry of tooth size, shape and even number. The internal environment may also change as development proceeds, causing the same genotype to be expressed differently at various stages of development. The retention of a child-like voice in castrated boys, and the acquisition of a masculine voice in women who produce pathologically high levels of male sex hormones, confirm that this change in expression of the genotype is a result of an alteration in hormone production.

INTERACTION BETWEEN GENES

If an individual is homozygous (or hemizygous) at a given locus for any allele, then the observed effect must be the effect of this allele alone. When an individual is heterozygous, possessing two different alleles at the same locus, the phenotype is dependent on the relationship between the alleles. If the two alleles are symbolized by A_1 and A_2, and in heterozygotes the observed effect is entirely that of A_2, then A_2 is completely dominant over A_1, and A_1 is completely recessive to A_2. All levels of dominance can occur from complete dominance of one allele over its partner to a situation of no dominance where each allele is expressed unaffected by the other.

Gene interaction can also occur between loci. For example, alleles at the 'secretor' locus in man control the presence or absence in the saliva and other body fluids of ABO blood group antigens, which are specified by another locus. In homozygotes for the dominant allele, *Se*, and in heterozygotes (*Sese*) blood group antigens appear in the saliva, but in homozygotes for the recessive non-secretor allele, *se*, they do not.

A further kind of interaction occurs when a particular gene has a large effect that can be modified by several other genes, each with a small effect. It is then convenient to refer to this particular gene as the major gene and the others as minor or modifying genes. The modifying genes are collectively known as the genetic background.

In animal experiments, crossing to different inbred strains is likely to result in variation of expression of a major gene since, because of their different origins, each strain probably has a unique complement of modifiers. For instance, hemizygotes for the X-linked gene 'tabby' in the mouse have, among their dental malformations, abnormalities of the incisors. Producing the hemizygous mutant genotype in five different inbred strains by appropriate crossing, resulted in an incidence of incisor abnormalities ranging from 11% for one strain to 86% for another. The results of crossing are not predictable but depend on random differences between strains that cannot be evaluated until after the crosses have been made.

PATTERNS OF INHERITANCE

Alleles are transmitted from one generation to the next in a predictable way. If a character is under genetic control, the distribution of the different forms of the character within families will be related to the pattern of inheritance of the alleles involved.

Inheritance of alleles at a single locus

AUTOSOMAL INHERITANCE

The nature of meiosis is such that for any one autosomal locus each gamete contains either the maternally derived allele, A_m, or the paternally derived allele, A_p. These two types of gamete are produced in equal numbers so that half the total gamete population contains A_m and the other half A_p. Any one gamete withdrawn at random therefore has the same chance of containing A_m as it does of containing A_p. Such chance situations can be defined in terms of probability (symbo-

Table 2.1. The four possible zygotic constitutions of maternally and paternally derived alleles, A_m and A_p, at an autosomal locus, and their probabilities of occurrence.

	Zygotic constitutions		Probabilities
	Allele from parent 1	Allele from parent 2	
1	A_m	A_m	$P_m \times P_m = 0.5 \times 0.5 = 0.25$
2	A_p	A_p	$P_p \times P_p = 0.5 \times 0.5 = 0.25$
3	A_m	A_p	$P_m \times P_p = 0.5 \times 0.5 = 0.25$
4	A_p	A_m	$P_p \times P_m = 0.5 \times 0.5 = 0.25$
		Total = 1.00	

lized by P), which is measured on a scale bounded by zero and one. The value $P = 1$ denotes absolute certainty, and $P = 0$ implies complete impossibility. In any situation the sum of the probabilities of all possible alternatives is equal to one. Thus, if P_m is the probability that a gamete withdrawn at random contains A_m, and P_p is the probability that it contains A_p, then $P_m = P_p = 0.5$ and $P_m + P_p = 1$.

When a particular event is dependent on the previous occurrence of more than one other event, the probability of this particular event occurring is equal to the product of the probabilities of each of the events upon which it is dependent. At fertilization, which can be regarded as the random union of two gametes, the probability that a zygote will receive maternally derived alleles from both parents is equal to $P_m^2 = 0.25$. This is one of a total of four possible zygotic constitutions, illustrated in Table 2.1, based on maternal or paternal origin of the allele contributed by each gamete.

Suppose that in Table 2.1 both parents are heterozygous for the same two alleles. There would then be only three possible zygotic constitutions based on genotype: the two homozygotes, each with a probability of 0.25, and a heterozygote, with a probability of 0.5. To illustrate how this comes about suppose that the maternally derived alleles of both parents are the same, say A_1, and that the paternally derived alleles of both parents are also the same, say A_2. Constitutions 1 and 2 in Table 2.1 then represent the two homozygotes, A_1A_1 and A_2A_2 respectively, and constitutions 3 and 4 show that there are two ways in which the heterozygote, A_1A_2, can be formed. Each of these has a probability of 0.25, so the total probability for heterozygotes is 0.5.

Consider now the different kinds of mating that can occur with respect to an autosomal locus at which there can be either allele A or allele a. Each parent can have one of the three possible genotypes AA, Aa or aa, and for each of the three genotypes that one parent can have the same three are available for the other. Thus there is a total of $3^2 = 9$ possible parental combinations. These are illustrated in Table 2.2. However, if it is not important which parent has which genotype, these nine combinations are reduced to six possible kinds of mating by duplication. The combinations are numbered in Table 2.2 to show where the duplications arise. The six kinds of mating and their outcomes are listed in Table 2.3. If P_A is the probability that a randomly withdrawn gamete contributed by a parent contains allele A, and P_a is the probability that it contains a, then for heterozygous parents $P_A = P_a = 0.5$, for AA homozygotes $P_A = 1$ and $P_a = 0$, and for aa homozygotes $P_A = 0$ and $P_a = 1$. The offspring genotype probabilities in Table 2.3 are simply the products of the probabilities for alleles contributed by the two parents and are a direct indication of the relative numbers of the different genotypes that can be expected among the progeny of each kind of mating. The transmission of different alleles to different offspring is known as segregation.

X-LINKED INHERITANCE

Gametes produced by males contain either an X-chromosome or a Y-chromosome, and these two types of gamete are produced in equal numbers. Gametes produced by females all contain an X-chromosome. The Y-chromosome of a male is therefore always inherited from his father and the

Table 2.2. The nine possible parental combinations with respect to a single autosomal locus with alleles A and a. The numbers in brackets show how these nine combinations are reduced to six possible kinds of mating by duplication. Pairs of cells containing the same number represent two different ways of forming the same kind of mating.

Genotype of parent 2	Genotype of parent 1		
	AA	Aa	aa
AA	(1) AA × AA	(2) AA × Aa	(3) AA × aa
Aa	(2) Aa × AA	(4) Aa × Aa	(5) Aa × aa
aa	(3) aa × AA	(5) aa × Aa	(6) aa × aa

X-chromosome is always inherited from his mother. Similarly, one of the X-chromosomes of a female is always the one carried by her father and the other comes from her mother. It follows that, whereas a mother's X-linked alleles are transmitted both to her daughters and sons, a father's X-linked alleles are transmitted only to his daughters, and through them to his granddaughters and grandsons. For example, the bleeding disorder haemophilia is caused by an X-linked mutant gene. Hemizygous mutant males show the disorder, but, since the mutant allele is almost completely recessive, heterozygous females are usually unaffected and are consequently known as carriers of the gene. A haemophiliac father transmits the mutant allele to all his daughters, who as a result are carriers and who, on average, pass it on to half their sons and half their daughters. These sons are haemophiliacs and these daughters are carriers.

Consider all the possible kinds of mating that can occur with respect to an X-linked locus at which there can be either allele A or allele a. There are then three possible genotypes for the female parent, AA, Aa and aa, and for each one there are two possible genotypes for the male parent, A and a. There is thus a total of $3 \times 2 = 6$ possible parental combinations. Unlike the autosomal case there is no duplication; male and female parents can never have the same X-linked genotype. Each of the six parental combinations therefore represents a completely different kind of mating. These matings and their outcomes are listed in Table 2.4. Again, the offspring probabilities are a direct indication of the relative numbers of the genotypes that can be expected among the progeny of the different kinds of mating.

Inheritance of characters controlled by a single locus

A knowledge of the genetic constitution of an individual allows predictions to be made about the

Table 2.3. The six possible kinds of mating (see Table 2.2) with respect to a single autosomal locus with alleles A and a. The probability that a randomly withdrawn gamete from each parent will contain a given allele is shown, as are the offspring genotype probabilities.

	Parent 1			Parent 2			Offspring genotype probabilities		
		Gamete probabilities			Gamete probabilities				
Mating	Genotype	A	a	Genotype	A	a	AA	Aa	aa
1	AA	1.0	0.0	AA	1.0	0.0	1.00	0.00	0.00
2	AA	1.0	0.0	Aa	0.5	0.5	0.50	0.50	0.00
3	AA	1.0	0.0	aa	0.0	1.0	0.00	1.00	0.00
4	Aa	0.5	0.5	Aa	0.5	0.5	0.25	0.50	0.25
5	Aa	0.5	0.5	aa	0.0	1.0	0.00	0.50	0.50
6	aa	0.0	1.0	aa	0.0	1.0	0.00	0.00	1.00

Table 2.4. The six possible kinds of mating with respect to an X-linked locus with alleles A and a. The probability that a randomly withdrawn gamete from each parent will contain a given allele is shown, as are the offspring genotype probabilities for the three possible genotypes of female offspring and two possible genotypes of male offspring.

	Female parent			Male parent			Offspring genotype probabilities				
		Gamete probabilities			Gamete probabilities		Females			Males	
Mating	Genotype	A	a	Genotype	A	a	AA	Aa	aa	A	a
1	AA	1.0	0.0	A	1.0	0.0	1.0	0.0	0.0	1.0	0.0
2	Aa	0.5	0.5	A	1.0	0.0	0.5	0.5	0.0	0.5	0.5
3	aa	0.0	1.0	A	1.0	0.0	0.0	1.0	0.0	0.0	1.0
4	AA	1.0	0.0	a	0.0	1.0	0.0	1.0	0.0	1.0	0.0
5	Aa	0.5	0.5	a	0.0	1.0	0.0	0.5	0.5	0.5	0.5
6	aa	0.0	1.0	a	0.0	1.0	0.0	0.0	1.0	0.0	1.0

types of offspring he produces. If there is no dominance or intermediate dominance all genotypes are phenotypically distinct, but if there is complete dominance of one allele over another it may only be possible to determine an individual's genotype through an examination of his progeny.

Table 2.5 lists the six possible kinds of mating with respect to a single autosomal locus with the alternative alleles A and a. The expected offspring segregation ratios are shown both for genotypes and also for phenotypes when A is completely dominant over a. The phenotype produced by genotypes AA and Aa is symbolized by A, and that produced by genotype aa is symbolized by a. Only three different parental combinations can be identified on the basis of phenotype alone, $A \times A$ (matings 1, 2 and 4), $A \times a$ (matings 3 and 5) and $a \times a$ (mating 6). All matings except 1 and 2 can, however, be distinguished through their offspring segregation ratios. Mating 4 has a different ratio from matings 1 and 2, and mating 3 has a different ratio from mating 5. Matings 1 and 2 can be separated by a second generation of matings of their A offspring with a individuals. Offspring of mating 1 are then partners in matings of type 3, whereas offspring of mating 2 contribute to some matings of type 3 and some of type 5. The transfer of dominant alleles from one generation to the next makes it possible for a dominantly controlled phenotype to occur in several successive generations of a family in the absence of inbreeding.

Table 2.6 shows a similar listing for a single X-linked locus. There are four parental combinations based on phenotype, $♀A \times ♂A$ (matings 1 and 2), $♀A \times ♂a$ (matings 4 and 5), $♀a \times ♂A$ (mating 3) and $♀a \times ♂a$ (mating 6). All matings are distinguishable on the basis of parental phenotype and offspring segregation ratio.

Table 2.5. The six possible kinds of mating with respect to an autosomal locus with alleles A and a. The expected ratios of the different types of offspring are shown for genotypes and for phenotypes when A is completely dominant over a.

	Genotypes		Phenotypes (A completely dominant over a)	
Mating	Parents	Offspring ratios AA : Aa : aa	Parents	Offspring ratios $A : a$
1	AA × AA	1 : 0 : 0	$A \times A$	1 : 0
2	AA × Aa	1 : 1 : 0	$A \times A$	1 : 0
3	AA × aa	0 : 1 : 0	$A \times a$	1 : 0
4	Aa × Aa	1 : 2 : 1	$A \times A$	3 : 1
5	Aa × aa	0 : 1 : 1	$A \times a$	1 : 1
6	aa × aa	0 : 0 : 1	$a \times a$	0 : 1

Table 2.6. The six possible kinds of mating with respect to an X-linked locus with alleles A and a. The expected ratios of the different types of offspring are shown for genotypes and for phenotypes when A is completely dominant over a.

	Genotypes			Phenotypes (A completely dominant over a)		
		Offspring ratios			Offspring ratios	
Mating	Parents ♀ × ♂	♀ AA : Aa : aa	♂ A : a	Parents ♀ × ♂	♀ *A* : *a*	♂ *A* : *a*
1	AA × A	1 : 0 : 0	1 : 0	*A* × *A*	1 : 0	1 : 0
2	Aa × A	1 : 1 : 0	1 : 1	*A* × *A*	1 : 0	1 : 1
3	aa × A	0 : 1 : 0	0 : 1	*a* × *A*	1 : 0	0 : 1
4	AA × a	0 : 1 : 0	1 : 0	*A* × *a*	1 : 0	1 : 0
5	Aa × a	0 : 1 : 1	1 : 1	*A* × *a*	1 : 1	1 : 1
6	aa × a	0 : 0 : 1	0 : 1	*a* × *a*	0 : 1	0 : 1

More complex situations

Many characters of interest are controlled by more than one locus, possibly with more than two alleles at each locus, but the theory behind the behaviour of such characters is nevertheless based on that described for two alleles at a single locus. When considering more than one locus the probability of any genotype occurring among the progeny of a particular mating is simply the product of the probabilities for the genotypes at each of the loci taken separately, and the complexity resulting from more than two alleles at a locus can be simplified by creating a number of two allele situations in which each allele is considered in relation to all the others. As an illustration of the enormous variety of expression that can occur for a character controlled by only a small number of loci with few alleles at each locus, it has been estimated that the wide range of human skin pigmentation among individuals of mixed Caucasian and black African ancestry can be accounted for by between three and six loci with two alleles per locus.

Demonstration of the mode of genetic control of a character

Genes are identified through variation in the characters they produce, and in particular by the distribution of different forms of each character within families. Theoretically, with unrestricted numbers of progeny from all kinds of mating, it is possible to determine the mode of genetic control of any character in great detail. However, because of restrictions of time and space in the laboratory, and because of difficulties associated with the collection of human data, it may only be practicable to make the fundamental distinction between control by a single locus and multifactorial control where many loci, or at least more than one, and an environmental component are involved. In the single locus case, analysis is based on the patterns of inheritance of the characters. The pattern of inheritance on the phenotypic level is compared with the theoretical expectations associated with different types of single locus control. The analysis of multifactorial control, on the other hand, is concerned with the size of the contributions of genetic and environmental sources to the observed variation between individuals, and rests on the degree of resemblance between relatives.

QUANTITATIVE AND POPULATION GENETICS

Quantitative and population genetics involve extensions of the basic principles already described. They permit the analysis of, respectively, the degree of resemblance between relatives and the behaviour of genes in populations rather than families.

Quantitative inheritance

When a character is under some degree of genetic control but no simple mode of inheritance can be demonstrated, the question of interest is: what proportion of the observed variation between individuals is due to genetic segregation and what proportion to environmental differences?

SOURCES OF VARIATION

Variation is expressed in terms of variance and variances are additive so that the total observed or phenotypic variance, V_P, is composed of a genetic component, V_G, and an environmental component, V_E, so that $V_P = V_G + V_E$. The genetic variance is itself made up of three components, the additive, dominance and interaction variances. Dominance and interaction effects are dependent on particular combinations of genes at the same and different loci respectively, and are therefore not inherited in a simple manner. However, they are likely to be small and, in addition, are difficult to estimate from human data. Human genetics is therefore usually concerned only with the additive genetic component of variance, V_A, which is the main genetic cause of resemblance between relatives. The proportion of the phenotypic variance taken up by the additive genetic component is known as the heritability and is symbolized by h^2. Thus, $h^2 = V_A/V_P$. Heritability is an expression of the reliance that can be placed on an individual's phenotype as an indication of the phenotype of his relatives. If, for a particular character, there is no dominance or interaction and the observed variation is entirely additive genetic in origin (that is $V_A = V_P$ and $h^2 = 1$), then the phenotype of an individual is, on average, equal to the mean phenotype of his parents.

As heritability is a ratio of one component of the observed variation to the total, changes in any component can affect its value. Thus, the heritability of the same character in genetically similar populations could have different values under different environmental conditions. Similarly, genetically different populations are likely to show variable heritabilities for the same character in the same environment. A specific heritability estimate therefore applies only to a particular population in its own environment.

ESTIMATION OF HERITABILITY

A common way of expressing the degree of resemblance between relatives for a continuously variable character is by a regression of offspring mean on midparent value, the mean of measurements of the character in the two parents of each family. If the two parental phenotypes are p_1 and p_2 then the midparent value is $\bar{P} = (p_1 + p_2)/2$. Suppose that $h^2 = 1$. All phenotypic variation has an additive genetic origin and the phenotype of an individual is an exact indication of his genotype. Since each individual receives, on average, half his genetic information from one parent and half from the other, the mean phenotype of offspring is $\bar{O} = (p_1/2) + (p_2/2)$. Therefore $\bar{O}$ tends to equal $\bar{P}$, and if $\bar{O}$ is plotted against $\bar{P}$ for several different families, the results average out as a straight line with a slope of one. When $h^2 = 1$, the regression of offspring on midparent value is $b_{\bar{O}\bar{P}} = 1$ and thus $h^2 = b_{\bar{O}\bar{P}}$. When $h^2 = 0$, and assuming that there is no environmental reason why offspring should resemble their parents, each offspring is equivalent to a randomly selected individual from the general population, and the offspring means of all families vary around the mean of the general population. The results of plotting $\bar{O}$ against $\bar{P}$ then average out as a straight line with zero slope. When $h^2 = 0$, $b_{\bar{O}\bar{P}} = 0$, so that in this case also $h^2 = b_{\bar{O}\bar{P}}$ (Fig. 2.4). The equivalence of h^2 and $b_{\bar{O}\bar{P}}$ can in fact be shown to apply for all values of h^2. Different methods of analysis are available for expressing the resemblance between other sorts of relatives.

Consider a quasicontinuous variable (Fig. 2.5). Individuals are classified as either affected or non-

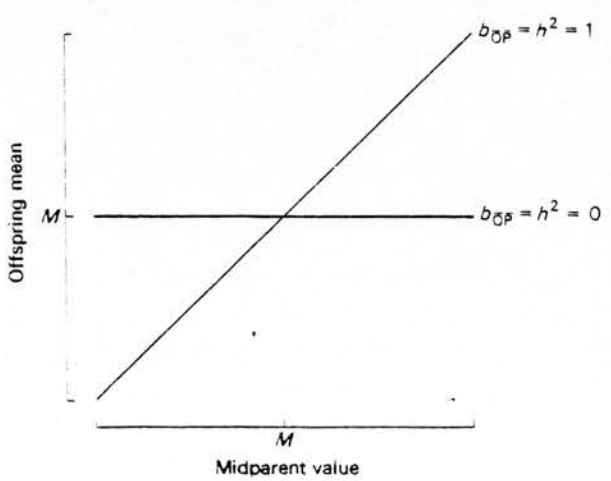


Fig. 2.4. The regression of offspring mean on midparent value. The two lines indicate the average relationships between parents and offspring for the two extremes of heritability. M is the mean value of the character in the population.

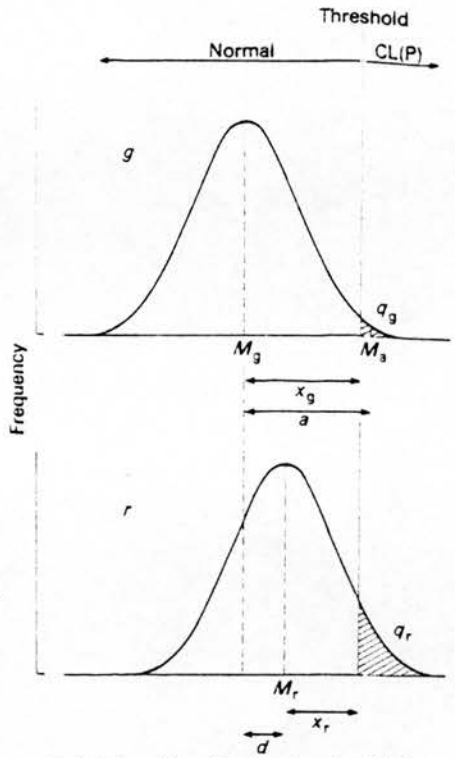


Fig. 2.5. Heritability estimation for a quasicontinuous or 'threshold' character, CL(P). Distributions of the general population, g, and of a group of relatives of affected individuals, r, are shown on an underlying continuous scale of disposition. The values of x_g and a, and x_r, are derived from tables of the normal distribution given the proportions q_g and q_r. The distance d, relative to a and the degree of relationship of the relatives used, provides the estimate of heritability.

affected. In human populations individuals affected by an abnormality or disease are usually in the minority and are the ones who attract attention. They are therefore often called index cases. As an example of a quasicontinuous variable consider cleft lip with or without a cleft of the palate, CL(P). The assumption is that for each individual a combination of genetic and environmental influences determines the level of disposition to develop the malformation. An individual whose level falls below the threshold is normal, whereas one whose level falls above it is affected. If CL(P) has no hereditary basis and is produced entirely by chance or environmental factors, then, provided all individuals are exposed to the same environment, a group of relatives of CL(P) individuals is equivalent to a random sample of the population with an incidence of the malformation approximating to that of the general population itself. On the other hand, if CL(P) is under some degree of genetic control the frequency of the malformation among relatives of index cases should be higher than among the general population. Thus, assuming that there is no environmental reason why relatives should be alike, d, the distance between M_r, the mean of a group of relatives of index cases, and M_g, the mean of the general population, is a measure of the degree to which CL(P) is a heritable condition (Fig. 2.5). This is simply the difference between x_g and x_r derived from applying the proportions q_g and q_r to tables. It must be assumed, though, that the variances of the general population and the group of relatives are the same.

The distance d must be considered in relation to a, the distance of the mean of affected individuals in the general population from the general population mean; and also to r, the degree of relationship of the relatives being used. The distance a is the maximum value that d can assume, and this can only occur if $h^2 = 1$ and if the relatives are monozygotic twins (genetically identical with their index cases, with r = 1). If the relatives used are first degree (full sibs, parents or children), second degree (aunts, uncles, nieces or nephews) or third degree relatives (first cousins) then r = 1/2, 1/4 and 1/8 respectively; these relatives have on average 1/2, 1/4 and 1/8 of their genes in common with their index case. The maximum values of d when using first, second and third degree relatives are accordingly a/2, a/4 and a/8, and these can only occur if $h^2 = 1$. The actual value of the heritability is simply the difference d expressed as a proportion of its maximum possible value. Thus $h^2 = d/ar$. Estimates of heritability for familial CL(P) from first, second and third degree relatives by this method are respectively 0.83, 0.78 and 0.81. These values suggest that the difference between familial CL(P) cases and normal individuals is probably largely (about 80%) due to the inheritance of different genes.

Genes in populations

Populations are described in terms of frequencies of different types of individual. Frequency, as used here, is expressed relative to the total number of individuals in the population. Used in this way, frequency is, like probability, measured on a scale bounded by zero and one, and the probability of withdrawing a particular type of individual from a population by random choice is equal to the frequency of that type in the population. Thus, in any situation, frequency and probability are numerically the same. From the genetic point of

view a population can be described in terms of phenotype frequencies, genotype frequencies and 'gene' frequencies (that is allele frequencies). Starting with gene frequencies it is a simple matter to derive genotype frequencies, and therefore phenotype frequencies, if certain assumptions are made about the population. Conversely, knowing the phenotype frequencies, it is possible to determine genotype and gene frequencies. The concepts involved in these determinations are embodied in the Hardy–Weinberg law.

THE HARDY–WEINBERG LAW

Suppose that A_1 and A_2 are two alleles at an autosomal locus, and that in a population the frequency of A_1 is p, the frequency of A_2 is q, and $p + q = 1$. From each individual taken at random the probability of withdrawing a gamete containing A_1 is equal to the frequency of A_1 ($= p$), and the probability of withdrawing a gamete containing A_2 is equal to the frequency of A_2 ($= q$). The results of random mating within the population, which is equivalent to the random union of gametes, are then as illustrated in Table 2.7. This shows that there are four possible zygotic constitutions with respect to parental origin of alleles but only three possible genotypes, as the heterozygote can be formed in two different ways. The frequencies of these genotypes, A_1A_1, A_1A_2 and A_2A_2, among the offspring are respectively p^2, $2pq$ and q^2.

For every A_1A_1 homozygote there are two A_1 alleles, for every A_2A_2 homozygote there are two A_2 alleles, and for every heterozygote there is one A_1 and one A_2 allele. Thus, if N offspring are produced after random mating there will be $N(2p^2 + 2pq)$ A_1 alleles and $N(2q^2 + 2pq)$ A_2 alleles. This is known as gene counting. As N individuals possess 2N alleles at an autosomal locus, and because $p + q = 1$, the frequencies of these alleles among the offspring are:

Frequency of A_1
$= N(2p^2 + 2pq)/2N$
$= p^2 + pq$
$= p^2 + p(1 - p) = p$

Frequency of A_2
$= N(2q^2 + 2pq)/2N$
$= q^2 + pq$
$= q^2 + q(1 - q) = q$

Thus the gene frequencies among the offspring of random mating are the same as in the parental generation.

Extending these principles over many generations it is clear that as long as random mating persists the gene frequencies will remain the same, and A_1A_1, A_1A_2 and A_2A_2 offspring will always be produced in frequencies p^2, $2pq$ and q^2 respectively. In such a situation the population is said to be in Hardy–Weinberg equilibrium. It should be stressed that the equilibrium genotype frequencies, which appear among the offspring of the first and subsequent generations of random mating, are quite independent of the genotype frequencies in the population from which the parents are drawn. They depend only on the gene frequencies.

It may also be helpful to consider the results of random mating in terms of genotypes only. Table 2.8 shows the frequencies of the nine possible parental combinations when the genotype frequencies have equilibrium values. These nine parental combinations are reduced to six kinds of mating (by duplication: they are numbered to show where the duplications arise.) The matings, their frequencies and their outcomes are listed in Table 2.9. The three genotypes of offspring, A_1A_1, A_1A_2 and A_2A_2, again appear in the frequencies p^2, $2pq$ and q^2 respectively.

Table 2.7. The four possible zygotic constitutions and three possible genotypes with respect to a single autosomal locus, and their frequencies assuming random mating. Alleles A_1 and A_2 have the frequencies p and q respectively, with $p + q = 1$.

	Zygotic constitutions				
	Allele from parent 1	Allele from parent 2	Frequency	Genotype	Genotype frequency
1	A_1	A_1	p^2	A_1A_1	p^2
2	A_1	A_2	pq	A_1A_2	$2pq$
3	A_2	A_1	pq		
4	A_2	A_2	q^2	A_2A_2	q^2

Total $= p^2 + 2pq + q^2 = 1$

Table 2.8. Frequencies of nine possible parental genotype combinations at an autosomal locus assuming random mating, reduced to six kinds of mating by duplication. Pairs of cells containing the same number in brackets represent two ways of producing the same kind of mating. The frequencies of the different kinds of mating, and their outcomes, are listed in Table 2.9.

Genotype of parent 2	Frequency	Genotype of parent 1 A_1A_1	A_1A_2	A_2A_2
		p^2	$2pq$	q^2
A_1A_1	p^2	(1) p^4	(2) $2p^3q$	(3) p^2q^2
A_1A_2	$2pq$	(2) $2p^3q$	(4) $4p^2q^2$	(5) $2pq^3$
A_2A_2	q^2	(3) p^2q^2	(5) $2pq^3$	(6) q^4

Similar theory can be applied to an X-linked locus, but account must naturally be taken of the difference in X-linked genotype between females and males.

FACTORS TENDING TO DISTURB HARDY–WEINBERG EQUILIBRIUM

The maintenance of Hardy–Weinberg equilibrium depends on random mating within the population. Random mating means that each individual of one sex has an equal chance of mating with each individual of the opposite sex, irrespective of genotype. Random mating, as already mentioned, is therefore equivalent to random union of gametes. If particular parental genotype combinations tend to occur in matings more frequently than expected by chance, the genotype frequencies among the offspring of the parental generation will be biased in favour of those produced by the more frequent kinds of mating.

Alleles transmitted from one generation to the next can be regarded as a sample of the alleles present in the parental generation. The larger the number of alleles transmitted the more confidence there can be that the frequencies among offspring are a good indication of the frequencies in the parental generation. The Hardy–Weinberg expectations are therefore dependent on population size. In small populations allele frequencies can change markedly from one generation to the next, purely by chance.

If the locus of interest has some control over fitness, then alleles associated with poor fitness will tend to be eliminated whereas others will tend to be present at progressively increasing frequencies. Hardy-Weinberg equilibrium is therefore only established in the absence of selection. Allele frequencies are also affected by the introduction of new allelic forms through mutation, and by the introduction or loss of alleles through migration into or out of the population.

Table 2.9. Six possible kinds of mating with respect to an autosomal locus. The two alleles A_1 and A_2 have the frequencies p and q respectively, with $p + q = 1$. The frequency of each kind of mating (from Table 2.8) and the frequencies of the three genotypes of offspring are based on the assumption of random mating.

	Mating	Mating frequency	Offspring genotype frequencies A_1A_1	A_1A_2	A_2A_2
1	$A_1A_1 \times A_1A_1$	p^4	p^4	0	0
2	$A_1A_1 \times A_1A_2$	$4p^3q$	$2p^3q$	$2p^3q$	0
3	$A_1A_1 \times A_2A_2$	$2p^2q^2$	0	$2p^2q^2$	0
4	$A_1A_2 \times A_1A_2$	$4p^2q^2$	p^2q^2	$2p^2q^2$	p^2q^2
5	$A_1A_2 \times A_2A_2$	$4pq^3$	0	$2pq^3$	$2pq^3$
6	$A_2A_2 \times A_2A_2$	q^4	0	0	q^4
	Total		p^2	$2pq$	q^2

These factors may also tend to upset Hardy-Weinberg equilibrium.

In spite of all this, statistical tests show that in most populations of reasonable size studied the genotype frequencies are in accordance with the Hardy–Weinberg expectations. Non-random mating, random sampling variation, differential selection, mutation and migration, although they probably occur to some extent in all populations, usually do not have large enough effects to disrupt Hardy–Weinberg equilibrium to a significant extent. With this in mind, the relationships between gene frequencies and genotype frequencies described by the Hardy–Weinberg law can be used to some advantage.

FURTHER READING

CAVALLI-SFORZA L.L. & BODMER W.F. (1971) *The Genetics of Human Populations*. San Francisco: W. H. Freeman.

EMERY A.E.H. (1979) *Elements of Medical Genetics*, 5th Edition. London: Churchill Livingstone.

FALCONER D.S. (1964) *Introduction to Quantitative Genetics*. London: Longman.

GRÜNEBERG H. (1963) *The Pathology of Development*. Oxford: Blackwell.

POOLE A.E., ed. (1975) Symposium on genetics. *Dental Clinics of North America* **19**. Number 1.

PRESCOTT G.H. & STEWART R.E., eds (1976) *Oral Facial Genetics*. St Louis: C.V. Mosby.

ROSS R.B. & JOHNSTON M.C. (1972) *Cleft Lip and Palate*. Baltimore: Williams and Wilkins.

SOFAER J.A. (1970) Dental morphologic variation and the Hardy–Weinberg law. *Journal of Dental Research* **49**, 1505.

SOFAER J.A. (1975) Genetic variation and tooth development. *British Medical Bulletin* **31**, 107.

SOFAER J.A. (1980) Single gene disorders. In *Oral Manifestations of Systemic Disease*, Chapter 2, eds. Mason D.K. & Jones H. London: W.B. Saunders.

STERN C. (1973) *Principles of Human Genetics*, 3rd Edition. San Francisco: W.H. Freeman.

WITKOP C.J. Jr., ed. (1962) *Genetics and Dental Health*. New York, Toronto and London: McGraw-Hill.

WITKOP C.J. Jr. (1965) Genetic disease of the oral cavity. In *Oral Pathology*, ed. Tieke R.W. New York, Toronto and London: McGraw-Hill.

RACIAL DIFFERENCES OF TOOTH MORPHOLOGY

A race is usually defined as a subdivision of a species formed by individuals who share common biological characteristics. Races can however be distinguished not only biologically but also from the cultural point of view, though the cultural differences are probably largely secondary to the biological ones and to differences of environment. The biological characteristics that have been used traditionally to distinguish the races of man are skin pigmentation, facial form and body build: characteristics that have been shown to be highly heritable. These have been chosen simply because they are conspicuous but heritable differences between races undoubtedly exist for a wide variety of less noticeable characteristics. The highly heritable nature of the biological characteristics distinguishing one race from another indicates that differences between races are fundamentally of genetic origin. Nevertheless, only a small proportion of all known human genetic variation appears to be responsible for racial differences. It has been estimated that, of the worldwide total of human genetic variation, about 80% occurs within races and only about 20% accounts for the biological differences between races.

It has long been known that minor morphological variation is superimposed on the basic shapes of human teeth. In common with the variation shown by other biological characteristics, much of this minor dental variation is found within races; different individuals of the same race often show different manifestations of each morphological variable. However, a proportion also occurs between races, so that individuals from a particular race tend to have a particular constellation of morphological features, with different constellations being typical of different races. In fact, minor differences of tooth shape have contributed to the characterization of the races of man and have been used to provide an indication of racial affinity between human populations. Both contemporary and past populations can be compared. The study of contemporary populations requires intraoral examination or, preferably, plaster casts of the teeth; that of past populations requires adequate skeletal or fossil remains. Nevertheless, it must be remembered that the use of a particular variant for the biological characterization of races is only valid if the variant is known to be largely under genetic control. This condition is probably fulfilled for most minor variants of tooth shape; in other words, individuals probably have particular dental morphological characteristics because they have inherited particular genes and not simply through chance or for some environmental reason. Direct evidence for the genetic control of a character comes from studies of resemblance between relatives, and for tooth morphology such studies are few and far between.

Even so, it is justifiable to use tooth morphology for studying racial affinity, provided it is borne in mind that the underlying basis for the observed variation is not yet fully understood. Affinity is measured by comparing the frequencies of different morphological variants in different groups. A similar percentage frequency of the same highly heritable variant form in two groups implies a high degree of affinity, whereas the greater the difference in frequency the lower the affinity and the greater the biological distance between the groups. Data for more than one variable can be combined to provide a single estimate of biological distance between two groups, and the larger the number of variables used the more reliable the estimate. A comparison of the pattern of racial affinities derived from a study of tooth morphology with the pattern based on other, better understood, biological criteria can provide a clue to the underlying basis of dental morphological variation. If the patterns are similar it is reasonable to infer that dental morphological variation is likely to have a high degree of genetic deter-

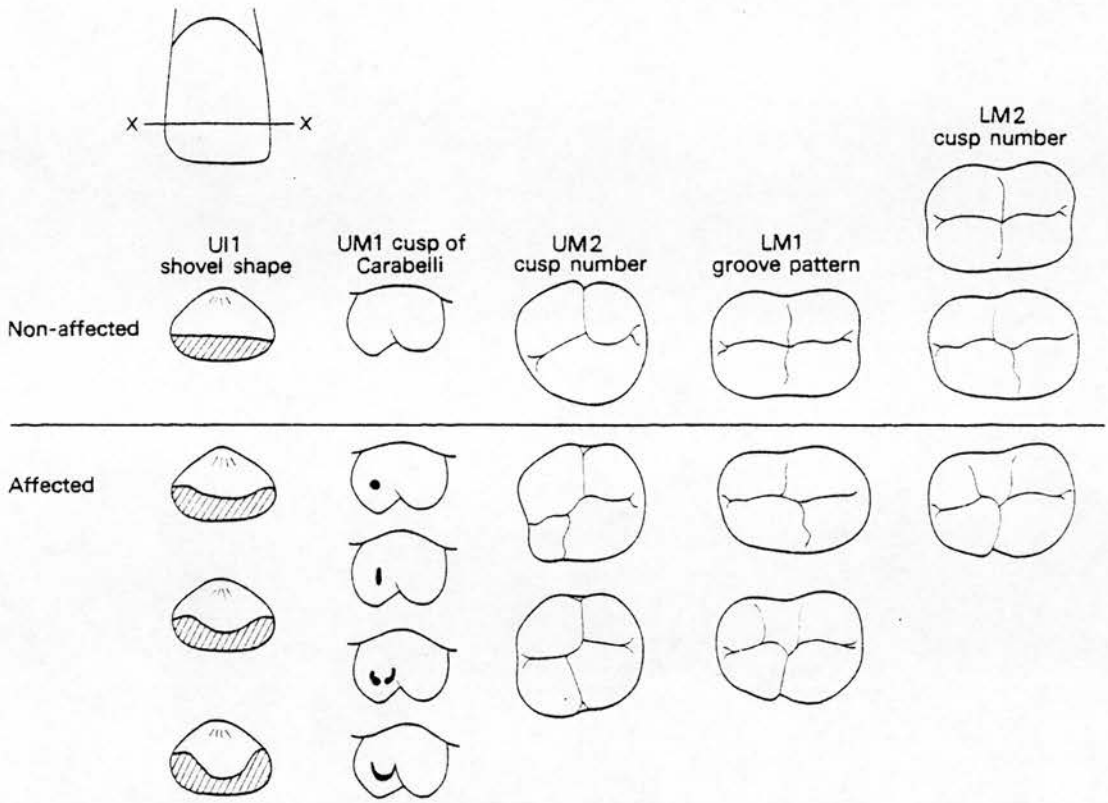


Fig. 5.33. Morphological variables on the upper central incisor (UI1), upper first and second molars (UM1 and UM2), and lower first and second molars (LM1 and LM2); and division of the range of variation of each into two categories, nonaffected and affected. Diagrams are modified from Lee G. T. R. and Goose D. H. (1972). The inheritance of dental traits in a Chinese population in the United Kingdom. *Journal of Medical Genetics* **9**, 336.

mination and can therefore make a useful contribution to the biological characterization of human populations.

There have been many studies of the frequency of variant forms for dental morphological characters in different populations. Five of the more commonly studied characters are illustrated in Fig. 5.33. They all appear to behave as quasi-continuous variables; that is, the variant form is either present or absent, but when present it can show different levels of expression from the lowest to the most extreme. For example, the upper second molar may have three cusps or four cusps but the fourth cusp, when present, varies in size. Some indication of the range of expression of the different characters is given in Fig. 5.33 but for the present purpose all the variables are regarded as all-or-none characters; in other words, a tooth is considered to be either non-affected or affected. The relevant characteristic of a population is the proportion of teeth affected.

The frequencies of the variant forms reported for different populations of the same racial group sometimes differ considerably. This must be partly because of real differences between samples taken from the different populations, but undoubtedly is also due to differences between the scoring criteria of different investigators. The most reliable estimate of the frequency of a variant in a given race is therefore the frequency of affected teeth in a group composed of as many samples as possible pooled together. Table 5.1 lists the lowest and highest frequency values found in the literature, each calculated from a single sample, and the pooled frequency estimate, calculated for teeth from several samples, for each of the five dental morphological characters in seven racial groups. The low and high values indicate the considerable range of variation in frequency reported for some of the characters in some racial groups. The pooled frequency estimates can be used to derive the most reliable pattern of affinities between different racial groups.

Many different statistical procedures, some very complex, have been used to combine observations on several characters to express biolog-

Table 5.1. The percentage frequency of affected teeth (%A) for five dental morphological characters in seven racial groups. For each character and each group the 'low' and 'high' values are the lowest and highest reported in the literature by different investigators, and the 'pooled' value is the frequency of affected teeth over several separately reported samples combined. N is the number of individuals on which each frequency is based. Sources of the data are given in SOFAER J.A., *et al.* (1972) Population studies on south-western Indian tribes. V. Tooth morphology as an indicator of biological distance. *American Journal of Physical Anthropology*, **37**, 357.

Racial group:		CAU Caucasian		SEM Semitic		NEG Negro	
Character	Estimate of %A	N	%A	N	%A	N	%A
UI1 Shovel shape	Low	100	17.0	137	41.5	264	16.6
	High	212	91.0	60	47.0	807	44.4
	Pooled	1833	40.5	197	43.2	1193	37.2
UM1 Cusp of Carabelli	Low	91	41.0	30	62.0	389	2.0
	High	140	85.7	30	93.0	274	57.7
	Pooled	3789	59.5	197	73.9	663	25.0
UM2 Cusp number	Low	53	58.0	137	30.5	78	100.0
	High	50	87.5	30	73.0	78	100.0
	Pooled	103	72.3	197	42.1	78	100.0
LM1 Groove pattern	Low	85	86.0	30	53.0	133	86.9
	High	75	96.0	137	70.4	49	100.0
	Pooled	221	91.6	197	65.7	182	90.4
LM2 Cusp number	Low	61	1.0	60	0	167	18.6
	High	356	14.0	137	7.0	69	53.7
	Pooled	611	11.0	197	4.9	285	28.2

Racial group:		PAC Pacific and Australia		ASI Asia		ESK Aleut and Eskimo		AMI American Indian	
Character	Estimate of %A	N	%A	N	%A	N	%A	N	%A
UI1 Shovel shape	Low	167	41.0	269	85.0	499	99.2	342	100.0
	High	59	88.1	259	97.7	267	100.0	342	100.0
	Pooled	1045	56.8	1817	92.8	766	99.5	342	100.0
UM1 Cusp of Carabelli	Low	67	19.4	339	31.9	60	13.3	41	12.0
	High	30	33.0	339	31.9	61	78.3	200	83.5
	Pooled	97	23.6	339	31.9	384	66.3	844	60.2
UM2 Cusp number	Low	53	69.8	887	84.6	91	65.7	53	66.2
	High	104	100.0	887	84.6	118	72.9	97	91.8
	Pooled	256	88.7	887	84.6	264	69.6	241	82.1
LM1 Groove pattern	Low	57	54.9	40	100.0	29	41.4	53	76.9
	High	20	100.0	40	100.0	30	97.0	55	100.0
	Pooled	77	66.6	40	100.0	202	88.2	270	95.1
LM2 Cusp number	Low	97	12.5	19	19.0	30	43.0	55	32.0
	High	20	48.0	21	31.0	58	66.1	53	72.0
	Pooled	232	24.6	40	25.3	124	57.4	197	60.4

ical distance between populations. Each has its own application. Probably the simplest that is suitable for the present purpose is the square root of the sum of squared differences of percentage frequency over all characters studied; that is, $\sqrt{\sum (P_1 - P_2)^2}$, where P_1 and P_2 are percentage frequencies of a particular variant in the two populations being compared. Distances calculated in this way, based on the five dental morphological variables illustrated in Fig. 5.33,

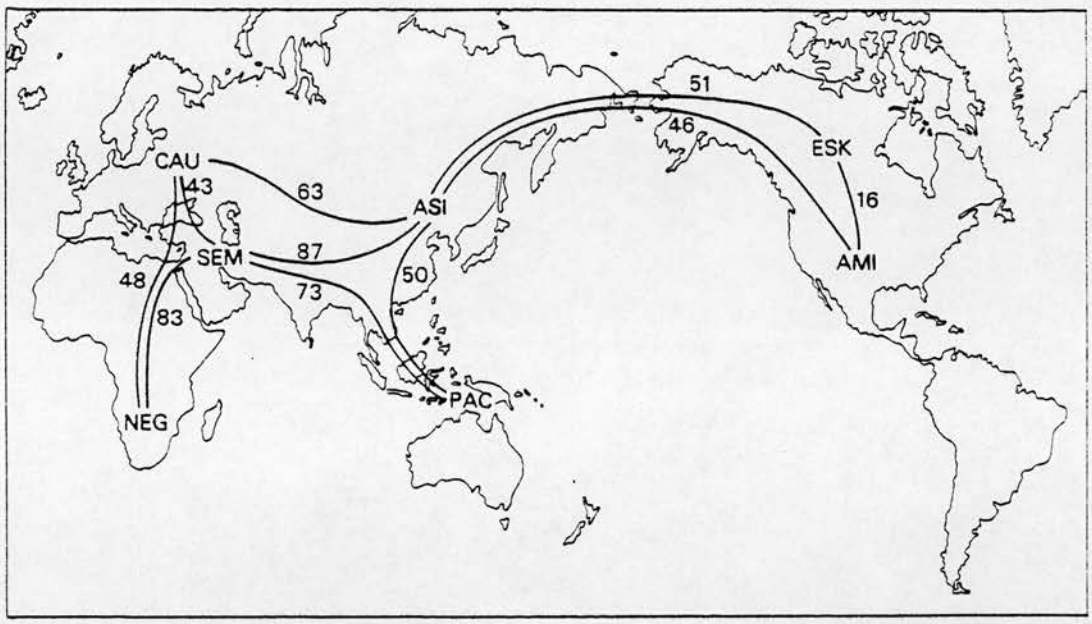


Fig. 5.34. A comparison between geographical distances along possible routes of migration and 'dental morphology distances' (given in figures against corresponding routes) separating neighbouring racial groups. The dental morphology distances are calculated from the frequencies of affected teeth for five dental morphological characters in several samples pooled for each racial group (see Table *5.1*). (CAU = Caucasian: SEM = Semitic: ASI = Asian: ESK = Aleut and Eskimo: AMI = American Indian: PAC = Pacific and Australia: NEG = Negroid)

are shown on a world map in Fig. 5.34. The position of each racial group is marked at approximately the geometric centre of the area from which samples contributing to the pooled frequency value were drawn. The geographical distances between racial groups, along possible routes of migration, are indications of the ease of admixture and consequently of genetic similarity. Provided there are no extreme geographical or cultural barriers between particular races, the shorter the geographical distance the shorter the biological distance.

Four 'triangular' relationships between neighbouring racial groups are shown on the map: CAU–SEM–NEG, CAU–ASI–SEM, SEM–ASI–PAC and ASI–ESK–AMI. For each of these the 'dental morphology distance' is least between the two geographically closest groups. For example, the dental morphology distance between the Caucasian and Semitic groups is less than that between either of these groups and the Asians. The dental morphology distances therefore show some degree of correspondence with the geographical distances, and so, assuming that biological distance is closely associated with geographical distance, the dental morphological variables considered here seem to be of some use for indicating biological relationships between populations.

It can be argued that racial differences of tooth morphology do reflect fundamental biological differences between races. However, there is still a need for family studies of tooth morphology to provide direct and more detailed information about the genetic control of human dental morphological variation. Until this information becomes available the precise value of dental morphological differences as indicators of biological distance, and the relative usefulness of different morphological variables for biological characterization, will be a matter for speculation.

FURTHER READING

DOWNER G.C. (1975) *Dental Morphology*. Bristol: Wright.

HARTY F.J. (1976) *Endodontics in Clinical Practice*. Bristol: Wright.

KRAUS B.S. & JORDAN R.E. (1965) *The Human Dentition before Birth*. Philadelphia: Lea & Febiger.

KRAUS B.S., JORDAN R.E. & ABRAMS L. (1969) *Dental Anatomy and Occlusion*. Baltimore: Williams and Wilkins.

SCOTT J.H. & SYMONS N.B.B. (1974) *Introduction to Dental Anatomy*, 7th Edition, Edinburgh: Livingstone.

WHEELER R.C. (1974) *A Textbook of Dental Anatomy and Physiology*, 5th Edition. London: Saunders.

8

Population genetics (Hardy-Weinberg equilibrium and factors affecting it)

J. A. Sofaer

An understanding of the behaviour in populations of the relatively rare mutant alleles responsible for single gene disorders can provide valuable insights into several aspects of medical genetics. For example, population genetics is applied in assessing the practicability of carrier screening programmes, it contributes to evidence for genetic heterogeneity and can be used to predict the possible effects of medical intervention on the incidence of genetic disease. A number of medical applications of population genetic principles are given later in this chapter, but first it is important to introduce the basic concepts involved.

Populations can be described in terms of frequencies of different types of individual or different types of allele at a given locus: phenotype frequencies, genotype frequencies and 'gene' frequencies (that is allele frequencies). Frequency is expressed relative to the total number of individuals or genes, and is therefore measured on a scale bounded by 0 and 1. Starting with gene frequencies it is a simple matter to derive genotype frequencies, and therefore phenotype frequencies, if certain assumptions are made about the population. Conversely, knowing the phenotype frequencies, it is possible to estimate genotype and gene frequencies. The concepts involved in these determinations were formulated independently by the English mathematician G.H. Hardy and the German physician W. Weinberg in 1908.

HARDY-WEINBERG EQUILIBRIUM

Two alleles at an autosomal locus

Suppose that A and a are two alleles at an autosomal locus, and that in a population the frequency of A is p, the frequency of a is q, and $p + q = 1$. For each gamete from each individual taken at random the probability of containing A is equal to the frequency of A ($= p$), and the probability of containing a is equal to the frequency of a ($= q$). The results of random mating (random with respect to genotype at the locus involved), which is equivalent to random union of gametes, are then as illustrated in Table 8.1. There are four possible zygotic constitutions with respect to parental origin of alleles but only three possible genotypes, as the heterozygote can be formed in two different ways. The frequencies of these genotypes, AA, Aa and aa, among the offspring are p^2, $2pq$ and q^2 respectively. This is simply an expansion of the expression $(p + q)^2$.

Table 8.1 The four possible zygotic constitutions and three possible genotypes with respect to a single autosomal locus, and their frequencies assuming random mating. Alleles A and a have the frequencies p and q respectively, with $p + q = 1$.

Zygotic constitution				
Male gamete	Female gamete	Frequency	Genotype	Genotype frequency
A	A	p^2	AA	p^2
A	a	pq	Aa	$2pq$
a	A	pq		
a	a	q^2	aa	q^2

Total genotype frequency $= p^2 + 2pq + q^2 = (p + q)^2 = 1$

For every AA homozygote there are two A alleles, for every aa homozygote there are two a alleles, and for every heterozygote there is one A and one a allele. Thus, if N offspring are produced after random mating there will be $N(2p^2 + 2pq)$ A alleles and $N(2q^2 + 2pq)$ a alleles. As N individuals possess $2N$ genes at an autosomal locus, and because $p + q = 1$, the frequencies of the different alleles among the offspring are:

$$\text{Frequency of } A = \frac{N(2p^2 + 2pq)}{2N}$$
$$= p^2 + pq$$
$$= p^2 + p(1 - p)$$
$$= p$$

$$\text{Frequency of } a = \frac{N(2q^2 + 2pq)}{2N}$$
$$= q^2 + pq$$
$$= q^2 + q(1 - q)$$
$$= q$$

Thus the gene frequencies among the offspring of random mating are the same as in the parental generation.

Extending these principles over many generations it is clear that as long as random mating persists the gene frequencies will remain the same, and AA, Aa and aa offspring will always be produced at the frequencies p^2, $2pq$ and q^2. In such a situation the population is said to be in Hardy-Weinberg equilibrium. It should however be stressed that the equilibrium genotype frequencies, which appear among the offspring of the first and subsequent generations of random mating, are quite independent of the parental genotype frequencies. They depend only on the gene frequencies.

It may also be helpful to consider the results of random mating in terms of genotypes. Table 8.2 shows the frequencies of the nine possible parental combinations when the genotype frequencies have equilibrium values. These nine parental combinations are reduced to six kinds of mating by duplication. The matings are numbered to show where the duplication arises. The matings, their frequencies and their outcomes are listed in Table 8.3. The three genotypes of offspring AA, Aa and aa, again appear in the frequencies p^2, $2pq$ and q^2 respectively.

Table 8.2 Frequencies of the nine possible parental genotype combinations for two alleles at an autosomal locus assuming random mating, reduced to six kinds of mating by duplication. The appearance of the same number in bold type twice indicates two ways of producing the same kind of mating. The frequencies and outcomes of the different kinds of mating are listed in Table 8.3.

Maternal genotype (and frequency)	Paternal genotype (and frequency) AA (p^2)	Aa ($2pq$)	aa (q^2)
AA (p^2)	**1** p^4	**2** $2p^3q$	**3** p^2q^2
Aa ($2pq$)	**2** $2p^3q$	**4** $4p^2q^2$	**5** $2pq^3$
aa (q^2)	**3** p^2q^2	**5** $2pq^3$	**6** q^4

Two alleles at an X-linked locus

For an X-linked locus the situation is rather different in that equilibrium is not reached after the first generation of random mating but is approached in an oscillatory manner over a number of generations. The reason for this is that the inheritance of X-chromosomes follows a different pattern in males and females. Males have only one X-chromosome which they inherit from their mothers, so the frequency of an X-linked gene in males is

Table 8.3 The six possible kinds of mating with respect to two alleles at an autosomal locus (see Table 8.2) and their offspring genotype frequencies.

Mating	Mating frequency	*Offspring genotype frequencies* AA	Aa	aa
1 $AA \times AA$	p^4	p^4	0	0
2 $AA \times Aa$	$4p^3q$	$2p^3q$	$2p^3q$	0
3 $AA \times aa$	$2p^2q^2$	0	$2p^2q^2$	0
4 $Aa \times Aa$	$4p^2q^2$	p^2q^2	$2p^2q^2$	p^2q^2
5 $Aa \times aa$	$4pq^3$	0	$2pq^3$	$2pq^3$
6 $aa \times aa$	q^4	0	0	q^4
Totals		$p^2(p^2 + 2pq + q^2) = p^2$	$2pq(p^2 + 2pq + q^2) = 2pq$	$q^2(p^2 + 2pq + q^2) = q^2$

Table 8.4 The six possible kinds of mating with respect to two alleles at an X-linked locus. The equilibrium frequencies of the alleles A and a are p and q respectively, with $p + q = 1$.

Paternal genotype (& freq)	Maternal genotype (& freq)	Mating frequency	*Offspring genotype frequencies* Male genotype A	a	Female genotype AA	Aa	aa
A (p)	AA(p^2)	p^3	p^3	0	p^3	0	0
A (p)	Aa ($2pq$)	$2p^2q$	p^2q	p^2q	p^2q	p^2q	0
A (p)	aa (q^2)	pq^2	0	pq^2	0	pq^2	0
a (q)	AA (p^2)	p^2q	p^2q	0	0	p^2q	0
a (q)	Aa ($2pq$)	$2pq^2$	pq^2	pq^2	0	pq^2	pq^2
a (q)	aa (q^2)	q^3	0	q^3	0	0	q^3
Totals:			$p(p^2+2pq+q^2) = p$	$q(p^2+2pq+q^2) = q$	$p^2(p+q) = p^2$	$2pq(p + q) = 2pq$	$q^2(p+q) = q^2$

equal to its frequency in females of the previous generation. Females have two X–chromosomes, one of paternal and the other of maternal origin. The frequency of an X–linked gene in females is therefore equal to the average of its frequencies in males and females of the previous generation. At equilibrium, gene frequency is the same in both sexes, the frequency of affected males is equal to that of the mutant allele, while the genotype frequencies for females are the same as for an autosomal locus. Table 8.4 shows that once equilibrium has been reached it is maintained over subsequent generations.

Multiple alleles

On the assumption of random mating, and by analogy with the two allele situation, the equilibrium frequencies of various genotypes at an autosomal locus where there are alleles A_1, A_2, . . . , A_n at frequencies p_1, p_2, . . . , p_n can be computed by expanding the expression $(p_1 + p_2 + \ldots + p_n)^2$. For an X–linked locus, just as in the two allele situation, the equilibrium frequencies of genotypes in males correspond with the allele frequencies, whereas in females they are the same as for an autosomal locus.

FACTORS TENDING TO DISTURB HARDY-WEINBERG EQUILIBRIUM

If a population is in Hardy-Weinberg equilibrium, the relationship between phenotype, genotype and gene frequencies can be used in a variety of ways in the practice of medical genetics (see below). However, the Hardy-Weinberg expectations rest on two fundamental assumptions: first, that mating is random; and second, that gene frequencies remain constant from one generation to the next. Any influence that encourages non-random mating or promotes changes of gene frequency could therefore result in deviation from the Hardy-Weinberg proportions. The significance of these influences will now be considered briefly.

Non-random mating

The two most important kinds of non-random mating are: (a) assortative mating, where like individuals preferentially select each other (positive assortative mating or homogamy) or unlike individuals preferentially select each other (negative assortative or disassortative mating); and (b) inbreeding, where related individuals mate with each other. In positive assortative mating, in so far as the common attributes of marriage partners are under genetic control, mating tends to occur between like genotypes. In inbreeding, because related individuals have more genes in common than unrelated individuals, mating also tends to occur between like genotypes. Both positive assortative mating and inbreeding therefore increase the level of homozygosity above the expected Hardy-Weinberg level. Nevertheless, if each genotype has the same probability of mating and producing progeny as any other, that is the same probability of passing genes on to the next generation, the allele frequencies will remain unchanged.

Positive assortative mating

Positive assortative mating for single gene differences is generally rare, but with the improvement of medical and social care for the disabled, the opportunity for marriage between two persons with the same disability, such as deafness or blindness, has increased. For recessive conditions, an increasing frequency of such marriages could result in a rise in the incidence of affected individuals in the population. However, because several different inherited disorders as well as nongenetic causes can result in each type of disability, the probability that the same recessive disorder is involved in a marriage between two individuals with the same disability is still rather low.

Consanguinity and inbreeding

Two individuals are considered to be consanguineous if they have at least one common ancestor no more remote than a great great grandparent. The offspring of consanguineous parents are, by definition, inbred. The level of inbreeding is expressed by the inbreeding coefficient, F. This is defined as the probability that an individual receives at a given locus two genes identical by descent. In other words, it is the probability that an individual is homozygous at the given locus because of inheriting the same gene from the same common ancestor through both parents.

It can be shown that each common ancestor contributes $\frac{1}{2}^{(n_1+n_2+1)}$ to the value of F, where n_1 and n_2 are the numbers of generations separating each of the two individuals involved in the consanguineous union from the common ancestor. Thus the coefficient of inbreeding can be given as $F = \Sigma\, \frac{1}{2}^{(n_1+n_2+1)}$, the summation being over common ancestors. For X–linked loci $F = \Sigma\, \frac{1}{2}^{n}$, where n is the number of females in each line of descent from a common ancestor in which there is no male to male succession. The level of consanguinity in a population as

Table 8.5 The coefficient of inbreeding, F, for various consanguineous matings.

Mating	F
Sibs	1/4
Uncle-niece, aunt-nephew	1/8
1st cousins	1/16
1st cousins once removed	1/32
2nd cousins	1/64
2nd cousins once removed	1/128
3rd cousins	1/256

a whole can be expressed by the average coefficient of inbreeding, $F = \Sigma p_i F_i$, where p_i is the proportion of marriages with inbreeding coefficient F_i. Table 8.5 lists various types of consanguineous mating and the corresponding F values.

Factors affecting gene frequency

Random genetic drift

In a large population, for loci that have no effect on viability or fertility, differences between individuals of different genotype in terms of the number of children produced tend to average out so that gene frequencies remain more or less stable. In a small population, the chance occurrence of a particular allele in parents with an unusually large (or unusually small) number of children can cause marked fluctuations of gene frequency from one generation to the next. This is a natural statistical consequence of sampling in a small population. The cumulative effect of these fluctuations over a number of generations, known as random genetic drift, can lead to complete extinction (gene frequency = 0) or fixation (gene frequency = 1) of an allele. However, because drift is a random process, changes of gene frequency from one generation to the next are not predictable. The precise effect of drift on Hardy-Weinberg equilibrium in any given situation is therefore difficult to assess.

Gene flow

The preferential introduction of particular alleles into a population can occur if gene frequencies among immigrating individuals are different from those in the population they are entering. This is known as gene flow, and its effect on Hardy-Weinberg equilibrium can perhaps be most easily understood in terms of a simple example of population admixture.

Suppose that a single event of admixture between two populations X and Y gives rise to the hybrid population Z, and that the frequencies of the only two alleles A and a at an autosomal locus in the three populations are p_X, p_Y and p_Z for A, and q_X, q_Y and q_Z for a. If w and $1\text{-}w$ are the relative contributions of populations X and Y respectively, then:

$$p_Z = wp_X + (1\text{-}w)p_Y \text{ and}$$
$$q_Z = wq_X + (1\text{-}w)\, q_Y$$

The frequency of heterozygotes after admixture but before mating, H_A, is then:

$$H_A = 2wp_Xq_X + 2(1-w)p_Yq_Y$$

and the equilibrium frequency of heterozygotes after one generation of random mating, H_E, is:

$$H_E = 2p_Zq_Z = 2\,[wp_X + (1-w)p_Y]\,[wq_X + (1-w)q_Y]$$

The difference between the equilibrium heterozygote frequency and that found after admixture but before random mating is therefore:

$$H_E - H_A = 2w(1-w)\,(p_X - p_Y)^2$$

This difference is always positive so that recent admixture always results in a deficiency of heterozygotes and therefore a tendency towards deviation from the expected Hardy-Weinberg proportions.

Mutation and selection

The majority of new mutations are deleterious and are therefore selected against. The frequencies of deleterious alleles in a population are thus dependent on two opposing forces, recurrent mutation which produces them, and selection which tends to eliminate them. If a population cannot be shown to deviate significantly from Hardy-Weinberg equilibrium, a balance between mutation and selection is assumed to exist.

Selection can be measured in terms of fitness, symbolised by f. This is the ability of an individual to contribute genes to the next generation and therefore depends on both viability and fertility, although normal viability with complete infertility is equivalent, in the genetic sense, to zero fitness. It is usual to express the fitness of mutant genotypes relative to that of normal individuals. Thus, if normal fitness is defined as 1, that of individuals with an inherited disorder can be given as $f = 1-s$, where s is the coefficient of selection, the proportional reduction in the contribution of the mutant genotype to the next generation.

For an autosomal recessive disorder caused by allele a:

		Frequency	
Genotype	Fitness	Before selection	After selection
AA	1	p^2	p^2
Aa	1	$2pq$	$2pq$
aa	$1-s$	q^2	$q^2(1-s)$
		1	$1 - sq^2$

The loss of sq^2 individuals as a proportion of the total occurs at each generation, and in those lost both genes are of the mutant type, a. The loss of a alleles as a proportion of all genes is therefore also sq^2. At equilibrium the gene frequency remains constant so that at each generation the loss of a alleles must be made good by new mutations. The mutation rate, μ, is the proportion of A alleles that mutate to a at each generation. The probability of mutation from A to a is therefore $p\mu$, which can be taken as μ, at each generation (q small, $p \simeq 1$). Therefore, if there is no change of gene frequency from one generation to the next, $\mu = sq^2$.

For a rare autosomal dominant disorder caused by allele A, homozygotes for which are too rare to be considered (p small, $q \simeq 1$):

		Frequency	
Genotype	Fitness	Before selection	After selection
aa	1	$q^2 \simeq 1 - 2p$	$1 - 2p$
Aa	$1 - s$	$2pq \simeq 2p$	$(1 - s)2p$
		1	$1 - 2ps$

The loss of $2ps$ individuals as a proportion of the total occurs at each generation, but in those lost only half the genes are of the mutant type, A. Therefore only ps mutant genes as a proportion of all genes are lost at each generation. The probability of mutation from a to A is $q\mu$, which can be taken as μ. Thus, if there is no change of gene frequency from one generation to the next, $\mu = ps$.

For a rare X-linked disorder caused by allele a, female homozygotes for which are too rare to be considered:

		Frequency	
Male genotype	Fitness	Before selection	After selection
A	1	p	p
a	$1 - s$	q	$q\,(1 - s)$
		1	$1 - sq$

The loss of sq males as a proportion of all males occurs at each generation, but only one third of all X-linked genes are carried by males. Therefore $sq/3$ a alleles as a proportion of all genes are lost at each generation. The probability of mutation from A to a is $p\mu$, which can be taken as μ (because $p \simeq 1$). Thus, if there is no change of gene frequency from one generation to the next, $\mu = sq/3$.

The high incidence of some recessive disorders in the face of a clear disadvantage to mutant homozygotes cannot always be accounted for by new mutations alone. In such situations the mutant allele may be maintained in the population through a selective advantage to heterozygotes. This can be represented for an autosomal locus as shown at the top of the next column. Because each AA homozygote has two A alleles and each heterozygote has one A allele, the proportion of A alleles lost is $2p^2s_1/(2p^2 + 2pq) = ps_1$. Similarly, the proportion of a alleles lost is qs_2. At equilibrium there is no change of gene frequency from one generation to the next. Therefore, disregarding the effect of new mutations, $ps_1 = qs_2$. When the recessive homozygote has zero fitness, that is when $s_2 = 1$, $ps_1 = q$ so that $s_1 = q/(1-q)$.

		Frequency	
Genotype	Fitness	Before selection	After selection
AA	$1-s_1$	p^2	$p^2(1-s_1)$
Aa	1	$2pq$	$2pq$
aa	$1-s_2$	q^2	$q^2(1-s_2)$
		1	$1 - p^2s_1 - q^2s_2$

THE VALIDITY OF HARDY-WEINBERG EQUILIBRIUM IN PRACTICE

Bearing in mind the considerable potential for disruption of equilibrium, it is important to be able to test populations for conformity with the Hardy-Weinberg expectations before the principles of equilibrium are applied in practice. This can be done as follows.

If x, y and z are the numbers of individuals of the three genotypes AA, Aa and aa respectively at an autosomal locus, and $x + y + z = N$, then, because N individuals possess $2N$ alleles at an autosomal locus, the frequencies of the two types of allele are $p = (2x + y)/2N$ for A and $q = (2z + y)/2N$ for a. This method of arriving at allele frequencies is known as gene counting. It is therefore a simple matter to calculate the expectation of p^2, $2pq$ and q^2 for the equilibrium genotype frequencies. The chi-square goodness of fit test can then be used to compare the observed numbers of each genotype (x, y, z) with those expected in Hardy-Weinberg equilibrium (p^2N, $2pqN$, q^2N). For this test, even though there are three categories, chi-square has one degree of freedom. This is because the expectations are derived from the observations through the computed allele frequencies, only one of which is sufficient to specify the expectations completely. The chi-square test can also be used to confirm equilibrium in the multiple allele situation where, in general, the degrees of freedom are the number of phenotypic classes minus the number of alleles. Examples of applications of the test to a two allele system and a three allele system can be found in Olaisen et al (1976) and Sorensen (1973).

The chi-square goodness of fit test indicates whether or not a population deviates significantly from Hardy-Weinberg equilibrium. Any such deviation can be expressed in terms of either a deficiency or an excess of heterozygotes. Three possible causes of heterozygote deficiency have already been outlined: positive assortative mating, inbreeding and admixture. Two possible causes of heterozygote excess have also been mentioned: heterozygote advantage and negative assortative mating. In addition, for some single gene conditions there may be a phenotypic overlap between homozygotes and het-

erozygotes, making the classification of phenotypes in terms of genotypes unreliable. Such errors in classification of phenotypes could result in spurious deviation from Hardy-Weinberg equilibrium. Other possible causes of deviation exist but need not be considered here. The problems involved in estimating deviations from Hardy-Weinberg equilibrium have been discussed more fully by Smith (1970).

Despite the potential for equilibrium to be disrupted, it is very unusual to find a population in which the observations deviate significantly from the Hardy-Weinberg expectations. However, failure to demonstrate deviation from the Hardy-Weinberg proportions does not imply that disrupting influences are not present. It simply indicates either that they are not sufficiently pronounced to be detected with the size of sample tested, or that more than one influence may be operating in opposite directions, tending to cancel each other out. It should also be pointed out that the chi-square goodness of fit test is not a sensitive test, so that any disruptive effect must be relatively large for a significant deviation to be demonstrated. On the other hand, it must be remembered that significant deviations will occur in a small proportion of tests, not for any biological reason, but simply because of the random nature of the sampling process.

The contribution of random sampling to observed deviations from equilibrium has recently been studied by comparing the results of numerous goodness of fit tests for three different genetic systems with the expected distribution of chi-square values, assuming that the Hardy-Weinberg assumptions are fully justified and that deviation results from random sampling alone. For two of the three systems the observed distribution of chi-square was significantly different from that expected, indicating that factors other than random sampling contributed to the observed deviations (Stevens & Fox, 1977). Nevertheless, because it is rare to find examples of significant deviation from the expected Hardy-Weinberg proportions it is accepted practice to assume that all large populations are in equilibrium unless there is evidence to the contrary. The expected genotype proportions can then be used to advantage in a variety of ways, some of which are outlined briefly below.

SOME APPLICATIONS OF THE HARDY-WEINBERG PRINCIPLE IN MEDICAL GENETICS

Estimating gene frequencies and genotype frequencies

Autosomal disorders

Consider again the two allele model summarised as follows:

		Population frequency	
Genotype	Number in sample	Dominant disorders	Recessive disorders
AA	x	$\simeq 0$	p^2
Aa	y	$2pq \simeq 2p$	$2pq$
aa	z	$q^2 \simeq 1-2p$	q^2
	N	1	1

For a rare dominant disorder caused by allele A (p small, $q \simeq 1$) the observed incidence of affected individuals, y/N, is equal to twice the gene frequency, p. Therefore $p = y/2N$.

For a completely recessive disorder caused by allele a, where the heterozygote cannot be distinguished from the normal homozygote, the observed incidence of affected individuals, z/N, is equal to the square of the gene frequency, q. Therefore $q = \sqrt{z/N}$. However, this estimate may be subject to a negative bias, particularly in small samples (Huether & Murphy, 1980). Having estimated q, and knowing that $p = 1-q$, the incidence of heterozygous carriers in the population can be calculated as $2pq$. Using this method, the frequency of the recessive allele for the Bloom syndrome among Ashkenazi Jews of Israel has been estimated as 0.0042, with a corresponding carrier incidence of approximately 1/120 (German et al, 1977). Similarly, the frequency of the recessive allele for chronic childhood spinal muscular atrophy in north-east England has been estimated as 0.0055 with a corresponding carrier incidence of 1/90 (Pearn, 1978). Alternatively, for a recessive disorder in which undetected lethality is suspected, the observed incidence of affected homozygotes can be compared with that expected on the basis of a known carrier frequency, y/N, since $y/N = 2pq = 2q(1-q)$, so that $2q^2 - 2q + y/N = 0$. This provides the solution $q = [1-\sqrt{1-(2y/N)}]/2$, from which the estimated incidence of affected conceptuses, q^2, can be derived (Severn, 1981).

For an incompletely recessive disorder where the heterozygote is recognisable, a better estimate of q can be obtained by gene counting from the equation $q = (2z + y)/2N$. The allele frequencies for erythrocyte glyoxalase types 1 and 2 have been found by this method to be 0.4512 and 0.5488 in a West German sample (Mauff et al, 1978).

X-linked disorders

For an X-linked disorder the incidence of affected males at equilibrium is equal to the population frequency, q, of the mutant allele. The incidence of carrier females might be expected to be $2pq$, or approximately $2q$ (because $p \simeq 1$). However, unlike autosomal recessive disorders where relatively few carriers are the offspring of affected individuals, in X-linked disorders the daughters of affected males constitute a potentially major pro-

portion of all carrier females. The fitness of affected individuals therefore has a much greater influence on the incidence of carriers in X–linked disorders than in autosomal recessive disorders.

For an X–linked disorder the complement of affected males in each generation is made up of those produced by new mutations and those who have inherited the mutant allele from carrier mothers. If the incidence of affected males and of carrier females at equilibrium are m and h respectively, then because half the male offspring of carriers are affected, $m = \mu + h/2$.

The complement of carrier females in each generation is made up of those produced by new mutations, those who have inherited the mutant allele from carrier mothers and those who are daughters of affected males. Therefore, because females have twice the chance of receiving a new mutation at an X–linked locus compared with males (provided the mutation rate is the same for both sexes), because half the female offspring of carriers are themselves carriers, and because the contribution made by daughters of affected males is dependent on the fitness, f, of affected males, $h = 2\mu + h/2 + fm$.
Substituting for μ:

$$h = 2(m - h/2) + h/2 + fm = m(4 + 2f)/3$$

The incidence of carriers for an X–linked disorder can therefore be expressed in terms of a ratio of carriers to affected males, given by the simple straight line relationship:

$$h/m = (4 + 2f)/3$$

A more complete approach to the analysis of equilibrium frequencies for X–linked disorders takes into account the possibility of reduced fitness in carriers and a difference of mutation rate between the sexes (Holloway & Smith, 1973).

Multiple allele frequencies

The estimation of multiple allele frequencies at an autosomal locus can be illustrated with reference to the ABO blood group system, where the frequencies of the A, B and O specificity producing alleles are p, q and r respectively. Then:

Phenotype	*Observed numbers*	*Genotype*	*Frequencies*
A	w	*AA* and *AO*	$p^2 + 2pr$
B	x	*BB* and *BO*	$q^2 + 2qr$
AB	y	*AB*	$2pq$
O	z	*OO*	r^2
	N		1

From this tabulation, $z/N = r^2$,
and $(w + z)/N = p^2 + 2pr + r^2 = (p + r)^2$
Therefore:

$$r = \sqrt{z/N}$$
$$p = \sqrt{(w + z)/N} - r$$
$$q = 1 - p - r$$

When all six genotypes produced by three alleles at a single autosomal locus can be distinguished, each allele frequency can be determined directly by gene counting, as for example in the red cell acid phosphatase system (Wiebecke & Brackebusch, 1974).

An allele frequency test of a genetic hypothesis

If a character exists in two distinct forms, individuals being classified as either 'affected' or 'non-affected', then the hypothesis that this character is controlled by two alleles at a single locus can be tested using the Hardy-Weinberg assumptions. Table 8.6 shows the different kinds of 'affected × affected' and 'affected × non-affected' matings, based on the hypothesis that there are two alleles, A and a, at frequencies p and q respectively, with $p + q = 1$ and with A completely dominant over a and producing the 'affected' phenotype. A prerequisite for the hypothesis is evidence that 'non-affected × non-affected' matings produce only 'non-affected' offspring. Mating and offspring frequencies are based on the assumptions of random mating and equal fitness of all genotypes. Taking the frequency of allele a as the square root of the observed incidence of 'non-affected' individuals, it is possible to derive the expected proportions of 'non-affected' individuals among the offspring of the two different classes of mating. The observed numbers of 'affected' and 'non-affected' offspring can then be compared with the numbers expected under single locus control by means of a chi-square test. However, even if no significant difference can be demonstrated there is a need for cautious interpretation, since alternative genetic and non-genetic hypotheses might fit the observations equally well. The ability to taste phenylthiocarbamide has been used to illustrate the application of the allele frequency test (Stern, 1973), and the test has been used to add weight to the contention that plasma paroxonase activity is controlled by two alleles at an autosomal locus (Playfer et al, 1976).

Inbreeding and recessive disorders

A population with average coefficient of inbreeding F can be regarded as equivalent to a hypothetical one in which a proportion, F, is fully inbred and the remainder, $1\text{-}F$, is bred at random. If alleles A and a at an autosomal locus are at frequencies p and q, with $p + q = 1$, then in the fully inbred part, where the probability of homozygosity through identity by descent is 1, the two types of homo-

Table 8.6 The different kinds of 'affected × affected' and 'affected × non-affected' matings, their frequencies and the frequencies of 'affected' and 'non-affected' offspring expected at Hardy-Weinberg equilibrium. Allele A is completely dominant over a and produces the 'affected' phenotype. The frequencies of the different kinds of mating are taken from Table 8.3.

	Mating	Mating frequency	*Offspring frequency* Affected	Non-affected
Affected × affected	$AA \times AA$	p^4	p^4	0
	$AA \times Aa$	$4p^3q$	$4p^3q$	0
	$Aa \times Aa$	$4p^2q^2$	$3p^2q^2$	p^2q^2
Total			$p^2(1+2q)$	p^2q^2
Relative			$1+2q$	q^2
Affected × non-affected	$AA \times aa$	$2p^2q^2$	$2p^2q^2$	0
	$Aa \times aa$	$4pq^3$	$2pq^3$	$2pq^3$
Total			$2pq^2$	$2pq^3$
Relative			1	q

Proportion of non-affected individuals among the offspring of:
affected × affected matings = $q^2/(1 + 2q + q^2) = [q/(1 + q)]^2$
affected × non-affected matings = $q/(1 + q)$

zygote are distributed simply as the corresponding gene frequencies. In the random bred part, all three genotypes are distributed according to the Hardy-Weinberg expectations. The overall genotype frequencies in the population are therefore:

	Frequencies		
Genotype	Inbred	Random bred	Total
AA	Fp	$(1-F)p^2$	$p^2 + Fpq$
Aa		$(1-F)2pq$	$2pq - 2Fpq$
aa	Fq	$(1-F)q^2$	$q^2 + Fpq$

When the incidence, m, of individuals affected by a rare recessive disorder is known, together with the average coefficient of inbreeding, F, it is possible to estimate the gene frequency, q, and the incidence of carriers, h, because:

$$m = q^2 + Fpq \simeq q^2 + Fq$$

and:

$$h = (1 - F)2pq$$

These equations have been used, for example, to calculate the gene frequency and carrier incidence for albinism among the Hopi indians of Arizona (Woolf & Dukepoo, 1969). It also follows from the relationship $m \simeq q^2 + Fq$ that the incidence of a recessive disorder is approximately twice the incidence expected under random mating when $F = q$, three times when $F = 2q$ and n times when $F = (n-1)q$. The highest levels of inbreeding recorded for human populations are in the region of $F = 0.04$, but such extreme values are very rare (Cavalli-Sforza & Bodmer, 1971).

Genetic heterogeneity in recessive disorders

The offspring of consanguineous marriages are more likely to be homozygous at any given locus than the offspring of unrelated parents. The frequency of consanguineous marriages among parents of individuals with recessive disorders is therefore higher than for the population at large. Furthermore, the rarer the recessive allele, the greater the frequency of consanguinity among parents of recessive homozygotes compared with the general population. This is illustrated in a recent study of a number of recessive disorders (Tchen et al, 1977). The majority of consanguinity can usually be attributed to first cousin marriages, so that the relationship between gene frequency and consanguinity can be expressed as:

$$q = \frac{C_G\,(1 - C_P)}{16C_P - C_GC_P - 15C_G}$$

where q is the recessive allele frequency at a single locus, and C_G and C_P are the frequencies of first cousin marriages in the general population and among parents of affected individuals respectively. (Dahlberg, 1948). Given C_G and C_P it is therefore a simple matter to arrive at an expected value for the incidence of a recessive disorder in the population, m_E, on the assumption that the disorder can only be produced by homozygosity at one particular locus, because, at equilibrium, $m_E \simeq q^2$.

If only one locus is indeed involved, the observed incidence of affected individuals in the population, m_O, should approximate to m_E. On the other hand, if the

disorder can be produced by homozygosity at any one of n loci, and assuming that the frequency of the recessive allele at each of these n loci is the same as in the single locus case, then C_G and C_P are unchanged, but the observed incidence of affected individuals in the population is greater than m_E by a factor of n. The ratio of the observed incidence of the disorder to that expected on the basis of single locus involvement therefore provides an estimate of the actual number of loci involved, the accuracy of this estimate depending on the relative contributions of the loci concerned.

The consanguinity approach to heterogeneity analysis in recessive disorders is mentioned by Stern (1973) in relation to albinism. Extensions of the method have been used to estimate the number of recessive genes involved in severe mental handicap (Dewey et al, 1965) and congenital deafness (Costeff & Dar, 1980).

Quantifying gene flow

If the frequency of a particular allele, a, at a given locus is known both for a hybrid population, Z, and for its two ancestral populations, X and Y, it is possible to estimate gene flow because $q_Z = wq_X + (1\text{-}w)q_Y$. Therefore w, the proportional contribution of population X to the hybrid population, can be given as:

$$w = (q_Z - q_Y)/(q_X - q_Y)$$

This formula has been used, for example, to quantify gene flow that has been occurring through racial admixture between blacks and whites in the United States (Reed, 1969) and in Cuba (Gonzalez et al, 1976).

Associated with the increase in heterozygosity that occurs with random mating after admixture, there is a decrease in homozygosity. The incidence of a recessive disorder after admixture but before random mating, which is simply the weighted mean of the incidence in the two ancestral populations, therefore falls once random mating has taken place.

Estimating mutation rates

For a disorder with an established mode of inheritance it is possible to estimate the mutation rate if the incidence of the condition and its fitness are known. Thus, if there is a balance between mutation and selection, and if the observed incidence of affected individuals is given as m, then:

for an autosomal recessive disorder $\mu = sq^2 = ms$
for an autosomal dominant disorder $\mu = ps = ms/2$
for an X-linked disorder (males only) $\mu = sq/3 = ms/3$

This approach has been used by Stevenson & Kerr (1967) to estimate mutation rates for a number of X-linked disorders. For autosomal dominant disorders, and for X-linked disorders where the heterozygote is recognisable, it may be possible to compare the expectations based on balance between mutation and selection with more direct estimates of mutation rate (Gardner, 1977; Davie & Emery, 1978).

Heterozygote advantage

The degree of heterozygote advantage required to maintain the incidence of a deleterious recessive disorder at a high level can be estimated if the gene frequency q, is known, because when the disorder confers zero fitness, $s_1 = q/(1 - q)$, where s_1 is the proportional reduction in the fitness of normal homozygotes relative to the fitness of heterozygotes. In fibrocystic disease and phenylketonuria, for example, the degree of heterozygote advantage required to maintain incidence at the present levels is 2–3 % and around 1 % respectively (Emery, 1976; Burdick, 1977). Attempts to confirm heterozygote advantage by direct estimation of fitness have been made for these two disorders by Knudson et al (1967) and Woolf et al (1975). However, as illustrated by Tay-Sachs disease, a deleterious allele can occur at high frequency for other reasons also (Spyropoulos et al, 1981).

Predicting the outcome of medical and social practices

The incidence of a genetic disorder may be decreased as a result of genetic counselling with consequent selective abortion or voluntary limitation of family size, or increased if the fitness of affected individuals is improved by medical treatment. The potential for reducing disease incidence through counselling depends on the proportion of mutant alleles inherited compared with those produced by new mutations. For dominant disorders, the only individuals able to transmit mutant alleles are those who have the condition themselves. Most dominant disorders have a rather low fitness, so the majority of affected individuals are recipients of new mutations. The potential for reducing the incidence of a dominant disorder through counselling is therefore generally less than the potential for increasing it by improving fitness. In recessive disorders, the majority of mutant alleles are contributed by carriers from the previous generation, most of whom have normal or near normal fitness and are not exposed to counselling. It may be possible to improve fitness for affected homozygotes, but because such individuals are rare compared with carriers this could lead to only a very slow increase in gene frequency. The potential for changing the frequency of a recessive disorder in either direction, at least over the next several generations, is therefore rather limited. The situation for X-linked disorders is intermediate, since mutant alleles are exposed to selection in males but can be transmitted by minimally affected carrier females.

The long term effect of any single medical or social practice on the incidence of an inherited disease can be

predicted by applying the basic principles of population genetics introduced in this chapter. For example, the effect of increasing the fitness of haemophiliacs has been discussed by Merritt (1975). However, it has been pointed out that predictions of this kind are unrealistic, both because a number of practices are operating together, some in opposite directions, and because the medical and social climates are changing continually. The net effect of current practices appears unlikely to cause any appreciable change in the incidence of genetic disorders in the foreseeable future, with the possible exception of dominant disorders in which fitness can be greatly improved by treatment. Nevertheless, it is important to monitor any changes that do take place and to assess the possible consequences of new practices as they are adopted (Holloway & Smith, 1975).

A fallacious application

It is appropriate to end by mentioning an application of population genetics that is not legitimate. Recently, attempts have been made to support the contention that various human characters are controlled by single autosomal loci, merely by applying the Hardy-Weinberg principle to the frequencies in populations of different manifestations of each character. The assumption has been that, for each character, three more or less arbitrarily defined levels of expression ('absent', 'moderately affected', 'maximally affected') correspond to the three genotypes produced by two alleles at a single locus. The agreement of the observed frequencies of these three hypothetical genotypes in the population with the Hardy-Weinberg expectations has then been taken as evidence of single locus control. The fallacy of this approach can be illustrated by applying the goodness of fit test in circumstances where the classification of individuals is known to have little or no biological basis. For example, the distribution of individuals over three categories according to the length of time since their last visit to the dentist ($\leq$ 1 year, 2–9 years, $\geq$ 10 years), from official American Dental Association statistics, has been shown to be consistent with single locus control of the character 'visiting the dentist', an obviously misleading conclusion (Sofaer, 1970). The testing for agreement of observed frequencies with the Hardy-Weinberg expectations in a population (rather than in families, as in an allele frequency test) is therefore only valid when it is applied to a known locus, identified through a study of related individuals. Its purpose then is to see whether or not a population is in equilibrium.

REFERENCES

Burdick A B 1977 Frequency of the gene for cystic fibrosis with a view of replacement and recognition effects and reproduction by homozygotes. Human Heredity 27: 366–371

*Cavalli-Sforza L L, Bodmer W F 1971 The genetics of human populations. Freeman, San Francisco

Costeff H, Dar H 1980 Consanguinity analysis of congenital deafness in northen Israel. American Journal of Human Genetics 32: 64–68

Dahlberg G 1948 Mathematical methods for population genetics. Karger, Basle; Interscience, New York

Davie A M, Emery A E H 1978 Estimation of proportion of new mutants among cases of Duchenne muscular dystrophy. Journal of Medical Genetics 15: 339–345

Dewey W J, Barrai I, Morton N E, Mi M P 1965 Recessive genes in severe mental defect. American Journal of Human Genetics 17: 237–256

*Emery A E H 1976 Methodology in medical genetics. Churchill Livingstone, Edinburgh

Gardner R J M 1977 A new estimate of the achondroplasia mutation rate. Clinical Genetics 11: 31–38

German J, Bloom D, Passarge E, Fried K, Goodman R M, Katzenellenbogen I et al 1977 Bloom's syndrome. VI. The disorder in Israel and an estimation of the gene frequency in the Ashkenazim. American Journal of Human Genetics 29: 553–562

Gonzalez R, Ballester J M, Estrada M, Lima F, Martinez G, Wade M et al 1976 A study of the genetical structure of the Cuban population: red cell and serum biochemical markers. American Journal of Human Genetics 28: 585–596

Holloway S M, Smith C 1973 Equilibrium frequencies in X-linked recessive disease. American Journal of Human Genetics 25: 388–396

Holloway S M, Smith C 1975 Effects of various medical and social practices on the frequency of genetic disorders. American Journal of Human Genetics 27: 614–627

Huether C A, Murphy E A 1980 Reduction of bias in estimating the frequency of recessive genes. American Journal of Human Genetics 32: 212–222

Knudson A G, Wayne L, Hallett W Y 1967 On the selective advantage of cystic fibrosis heterozygotes. American Journal of Human Genetics 19: 388–392

Mauff G, Wachauf P, Schaal K P, Pulverer G 1978 GLO I and Bf phenotype distribution in a West German population sample. Human Heredity 28: 431–434

Merritt A D 1975 Population genetics and hemophilia–implications of mutation and carrier recognition. Annals of the New York Academy of Sciences 240: 121–131

Olaisen B, Teisberg P, Jonassen R 1976 ESD polymorphism in Norway. Human Genetics 34: 63–64

Pearn J 1978 Incidence, prevalence and gene frequency studies of chronic childhood spinal muscular atrophy. Journal of Medical Genetics 15: 409–413

Playfer J R, Eze L C, Bullen M F, Evans D A P 1976 Genetic polymorphism and interethnic variability of plasma paroxonase activity. Journal of Medical Genetics 13: 337–342

Reed T E 1969 Caucasian genes in American negroes. Science 165: 762–768

Severn D J 1981 Thalassaemia and Haldane's approximation. Lancet ii: 89

Smith C A B 1970 A note on testing the Hardy-Weinberg law. Annals of Human Genetics, London 33: 377–383

Sofaer J A 1970 Dental morphologic variation and the Hardy-Weinberg law. Journal of Dental Research 49: 1505–1508

Sorensen S A 1973 Human red cell acid phosphatase polymorphism. Population and family studies in Denmark. Human Heredity 23: 470–481

Spyropoulos B, Moens P B, Davidson J, Lowden J A 1981 Heterozygote advantage in Tay-Sachs carriers? American Journal of Human Genetics 33: 375–380

Stern C 1973 Principles of human genetics, 3rd edn. Freeman, San Francisco

Stevens A, Fox W 1977 χ^2 for agreement with Hardy-Weinberg proportions. Human Heredity 27: 247–250

*General reference

Stevenson A C, Kerr C B 1967 On the distribution of frequencies of mutation to genes determining harmful traits in man. Mutation Research 4: 339–352

Tchen P, Bois E, Feingold J, Feingold N, Kaplan J 1977 Inbreeding in recessive disease. Human Genetics 38: 163–167

Wiebecke D, Brackebusch H D 1974 Studies on the polymorphism of the red cell acid phosphatase in the population of northern Bavaria (*Germany*). Humangenetik 23: 230–232

Woolf C M, Dukepoo F C 1969 Hopi indians, inbreeding and albinism. Science 164: 30–37

Woolf L I, McBean M S, Woolf F M, Cahalane S F 1975 Phenylketonuria as a balanced polymorphism: the nature of heterozygote advantage. Annals of Human Genetics, London 38: 461–469

11

Twins

J. A. Sofaer

The value of twin studies rests on the fact that there are two different types of twin pair: monozygous (monozygotic, MZ, identical), where both members of a pair are derived from a single fertilised egg and can therefore be presumed to be genetically identical; and dizygous (dizygotic, DZ, fraternal), where members of a pair develop from different fertilised eggs and are thus comparable to normal sibs. Differences between members of a monozygous pair are consequently entirely non-genetic (environmental) in origin, whereas differences between members of a dizygous pair are due to a combination of genetic and environmental influences. Other types of twinning are possible but are likely to occur with a negligible incidence.

The relative incidence of MZ and DZ twinning can be estimated using the method proposed by Weinberg. All MZ pairs, by definition, must be like-sexed. In DZ pairs the sexes are presumably combined at random so that half the DZ pairs should be like-sexed and half unlike-sexed (¼ ♀♀, ½ ♀♂, ¼ ♂♂), assuming a sex ratio of unity. Thus, if L and U are the numbers of like-sexed and unlike-sexed pairs observed, the estimated proportions of MZ and DZ pairs are respectively $(L-U)/(L+U)$ and $2U/(L+U)$.

The random combination of sexes in DZ twins has however been questioned because of evidence for a preponderance of like-sexed pairs (James, 1976, 1979). Such a preponderance would lead to an overestimate of the proportion of MZ twinning and a corresponding underestimate for DZ twinning. Nevertheless, pooled data from the literature have failed to support the non-random association of sexes (Bulmer, 1976). An appropriate adjustment can be made for deviation from a sex ratio of unity, based on observations in the population under study, but the effect is likely to be negligible.

The incidence of MZ twinning appears to be stable throughout the world, being around 4/1000 births. By comparison, the incidence of DZ twinning is very variable, ranging from around 3/1000 in Oriental populations, through around 7/1000 among Caucasians, up to around 20/1000 births for some Negro populations. The incidence of all twinning among Nigerians is particularly high, being in the region of 4.5% (Morton et al, 1967). Monozygous twinning, which presumably results from chance developmental variation, is also little affected by maternal status, whereas DZ twinning, which has its basis in multiple ovulations, has been shown to increase with maternal age and parity (Bulmer, 1970; Elwood, 1978), and to be influenced by maternal genotype (White & Wyshak, 1964; Morton et al, 1967) and secular environmental changes (British Medical Journal, 1976; Brackenridge, 1977; Nielsen et al, 1978).

ZYGOSITY

A prerequisite to the use of twins in studying the genetic contribution to observed variation is a reliable diagnosis of zygosity, the accurate classification of twin pairs as either MZ or DZ. This, of course, need only be considered for like-sexed pairs, since unlike-sexed pairs are always DZ. Different approaches are available. First, knowledge of the configuration of the fetal membranes can provide some information. Monochorionic pairs are invariably MZ whereas dichorionic pairs are only largely DZ, the dichorionic group including a proportion of MZ pairs also. Second, reciprocal skin grafting can be used. This is a potentially powerful, although impractical, means of zygosity diagnosis simply because the large number of polymorphic loci contributing to tissue compatibility is unlikely to allow DZ twins to have compatible genotypes at all these loci by chance. However, the occasional occurrence of a vascular anastomosis between the early fetal circulations of fraternal twins, and the possible consequent development of mutual immunological tolerance, suggests that even this method of zygosity diagnosis could be subject to occasional error. The third and most usual approach involves an assessment of similarity in terms of a number of different inherited characteristics, independent of the traits the study has been designed to investigate.

Similarity in terms of simple visual criteria may provide an acceptable level of zygosity diagnosis under some circumstances when used by an experienced obser-

ver, and questionnaires to assess similarity, directed towards either twins themselves or their parents,' have been found to be remarkably reliable (Cohen et al, 1975; Kasriel & Eaves, 1976). Nevertheless, for research purposes more objective information is desirable (Carter-Saltzman & Scarr, 1977). Three sources of this information are available: the population incidence of monozygosity and dizygosity; phenotypes/genotypes for characters controlled by single loci; and within pair differences for quantitative characters, particularly those with high heritabilities.

Prior probabilities of monozygosity and dizygosity among like-sexed pairs

Given a like-sexed pair but no additional information, the prior probabilities of dizygosity and monozygosity can be expressed in terms of the incidence of dizygosity and monozygosity among like-sexed pairs in the population under study, approximately U/L and $(L-U)/L$ respectively. Given a like-sexed pair the prior probability of dizygosity relative to the probability of monozygosity, the ratio R, is then: $R = U/(L-U)$.

Single locus polymorphisms

Phenotype or genotype information for a number of polymorphic loci controlling such characters as blood groups, serum proteins or tissue enzymes serves either to confirm dizygosity (once a genotype difference has been detected) or to decrease the probability of dizygosity (with increasing number of loci for which identical phenotypes/genotypes have been demonstrated). Given phenotypic/genotypic identity, the probability of dizygosity relative to the probability of monozygosity, the ratio s, can be calculated if either the parental genotypes or the population gene frequencies are known.

The relative probability of dizygosity given parental genotypes

Table 11.1 shows how the relative probability of dizygosity can be calculated from observations of polymorphisms when the parental genotypes are known and when at least one parent is heterozygous. Three different situations are illustrated. At locus 1, one parent is heterozygous and the other homozygous, but at loci 2 and 3 both parents are heterozygous. At locus 1 the heterozygote, *Aa*, is distinguishable from the homozygote, *AA*, but at locus 3 the allele *C* is dominant.

The relative probability of dizygosity from population gene frequencies

Table 11.2 shows how the relative probability of dizygosity can be calculated using gene frequency data when the parental genotypes are unknown. For each observed twin genotype the probability of dizygosity relative to the probability of monozygosity, the ratio s, is expressed in terms of p, the population frequency of allele A. For each twin genotype, s is therefore a different function of gene frequency (Gaines & Elston, 1969).

When there are more than two alleles at a locus calculation becomes much more complex. However, tables of probabilities based on gene frequency data have been constructed for the more commonly tested polymorphisms (Smith & Penrose, 1955; Smith et al, 1961).

Quantitative characters

For quantitative characters such as height, finger ridge count, *atd* angle and so on, the relative probability of dizygosity can be derived by reference to previously observed distributions of within twin pair differences for known MZ and DZ pairs. This is illustrated in Table 11.3. Given monozygosity, the probability of an observed difference of magnitude i is therefore m_i/M, and given dizygosity, the probability of an observed difference of the same magnitude is d_i/D. Given an observed difference of magnitude i, but no other information, the probability of dizygosity relative to the probability of monozygosity, the ratio s, is therefore $s = d_iM/m_iD$.

Published tables are available listing the relative probability of dizygosity for given ranges of observed within pair differences for a number of the more commonly used quantitative characters (Smith & Penrose, 1955; Smith et al, 1961).

The overall probability of dizygosity

The overall probability of dizygosity relative to the overall probability of monozygosity, the ratio S, taking into account the prior probability of dizygosity among like-sexed pairs in the general population and the relative probabilities of dizygosity from all single locus poly-

Table 11.1 The probability of dizygosity relative to the probability of monozygosity, the ratio s, from observations for three autosomal loci at which parental genotypes are known. At locus 1 the heterozygote is recognisable but at locus 3 the allele *C* is dominant.

Locus	Known parental genotypes	Observed twin genotype	*Probability of observed genotype* in 1 zygote	in 2 zygotes	s
1	$AA \times Aa$	AA	1/2	1/4	0.5
2	$Bb \times Bb$	bb	1/4	1/16	0.25
3	$Cc \times Cc$	CC or Cc	3/4	9/16	0.75

Table 11.2 The probability of dizygosity relative to the probability of monozygosity, the ratio s, for three different twin autosomal genotypes when the parental genotypes are unknown, based on the assumption of random mating. The population frequencies of alleles A and a are p and q respectively, with $p + q = 1$.

Observed twin genotype	Possible parental genotypes	Mating frequency	*Probability of observed genotype* in 1 zygote	in 2 zygotes	s
AA	$AA \times AA$	p^4	p^4	p^4	
	$AA \times Aa$	$4p^3q$	$2p^3q$	p^3q	
	$Aa \times Aa$	$4p^2q^2$	p^2q^2	$p^2q^2/4$	
	Total:		p^2	$p^2(1 + p)^2/4$	$(1 + p)^2/4$
Aa	$AA \times Aa$	$4p^3q$	$2p^3q$	p^3q	
	$AA \times aa$	$2p^2q^2$	$2p^2q^2$	$2p^2q^2$	
	$Aa \times Aa$	$4p^2q^2$	$2p^2q^2$	p^2q^2	
	$Aa \times aa$	$4pq^3$	$2pq^3$	pq^3	
	Total:		$2pq$	$pq(1 + p - p^2)$	$(1 + p - p^2)/2$
AA or Aa	$AA \times AA$	p^4	p^4	p^4	
	$AA \times Aa$	$4p^3q$	$4p^3q$	$4p^3q$	
	$AA \times aa$	$2p^2q^2$	$2p^2q^2$	$2p^2q^2$	
	$Aa \times Aa$	$4p^2q^2$	$3p^2q^2$	$9p^2q^2/4$	
	$Aa \times aa$	$4pq^3$	$2pq^3$	pq^3	
	Total:		$p(2 - p)$	$p(4 + 5p - 6p^2 + p^3)/4$	$\dfrac{4 + 5p - 6p^2 + p^3}{4(2 - p)}$

Table 11.3 The distribution of observed differences within pairs of twins of known zygosity for a quantitative character, expressed in terms of arbitrary units on the appropriate continuous scale. The numbers of twin pairs showing each class of difference are given by m and d.

Difference between measurements in arbitrary units	0	1	2	3	. .	n	Total
MZ pairs	m_0	m_1	m_2	m_3		m_n	M
DZ pairs	d_0	d_1	d_2	d_3		d_n	D

morphisms and quantitative characters scored, is simply the product of the individual relative probabilities. Thus, based on n traits, $S = Rs_1s_2s_3 \ldots s_n$. The probability of dizygosity, P_{DZ}, allowing that twins can be only MZ or DZ, is therefore $P_{DZ} = S/(1 + S)$, and the probability of monozygosity, P_{MZ}, is similarly $P_{MZ} = 1/(1 + S) = 1 - P_{DZ}$.

THE ANALYSIS OF TWIN DATA

The resemblance between twins can be expressed either in terms of an all-or-none character, such as a congenital malformation or disease state, or in terms of a continuous variable, such as intraocular pressure or the concentration of a serum constituent. Different approaches are required for the two different kinds of trait, but the ultimate goal is to use resemblance to assess the contribution of heredity to the observed variation. In addition, the families of MZ twins provide a unique opportunity to estimate certain components of variation, the magnitude of which might otherwise remain a matter for speculation.

Concordance

The study of resemblance between twins for all-or-none characters involves comparing the number of pairs that are concordant, where both members are affected (++), with the number of pairs that are discordant, where only one member is affected (+−).

The pairwise concordance rate, C_w, is defined as the proportion of concordant twin pairs among all those which include at least one affected member, so that $C_w = C/(C + D)$, where C and D are the numbers of ++

and +− pairs respectively. A more acceptable measure of concordance is the proband concordance rate, C_p, which takes account of ascertainment. It is equivalent to the incidence of the trait among co-twins of previously ascertained cases, so that ++ pairs in which both members have been ascertained independently are counted twice. The proband concordance rate is therefore given as $C_p = (C + C')/(C + D + C')$, where C is the total number of ++ pairs, C' is the number of ++ pairs in which the two affected members have been ascertained independently, and D is the number of +− pairs. When ascertainment is complete, $C = C'$, so that $C_p = 2C/(2C+D)$.

The significance of a difference in concordance between MZ and DZ twins can be tested by means of a 2 × 2 contingency chi-square, as has been done, for example, with allergic disorders (Lubs, 1972); but such a test is only able to indicate whether MZ twins are significantly more alike than DZ twins with respect to the character studied and cannot provide a quantitative estimate of the genetic contribution to resemblance. It is however possible to derive an estimate of heritability from a concordance rate using the assumptions involved in the multifactorial threshold model (Falconer, 1965). The incidence of the trait in the general population, q_g, establishes the distance, x_g, of the mean of the general population from the threshold on the presumed underlying scale of continuous variation, the scale of 'liability', and the proband concordance rate, q_{MZ} or q_{DZ}, establishes the distance, x_{MZ} or x_{DZ}, of the mean of the distribution of co-twins (either MZ or DZ) from the threshold. A simple approximate estimate of the correlation, r, between twins is then given by $r_{MZ} = (x_g - x_{MZ})/a$ and $r_{DZ} = (x_g - x_{DZ})/a$ for MZ and DZ pairs respectively, where a is the distance between the overall mean of the general population and the mean of affected individuals in the general population. Values for x and a are given in tables in standard deviation units against the appropriate values of q (Falconer, 1965; Emery, 1976).

These correlations estimate the proportion of the total phenotypic variance, V_P, which is common to members of a twin pair, so that $r_{MZ} = (V_A + V_D + V_C)/V_P$ and $r_{DZ} = (\frac{1}{2}V_A + \frac{1}{4}V_D + V_C)/V_P$, where V_A is the additive genetic variance, V_D the dominance variance and V_C the common environmental variance (Falconer, 1960). The variance due to epistatic interaction between loci is regarded as negligible. Thus, if $V_D = 0$ and $V_C = 0$, the heritability is estimated simply as $h^2 = V_A/V_P = r_{MZ} = 2r_{DZ}$. Non-genetic familial effects are however likely to make a significant contribution to resemblance between twins; but such effects can be eliminated in the analysis, assuming the same common environmental variance for MZ and DZ pairs, by using the difference between the MZ and DZ correlations, when $2(r_{MZ} - r_{DZ}) = (V_A + \frac{1}{2}V_D)/V_P$. Thus, if $V_D = 0$, $h^2 = V_A/V_P = 2(r_{MZ} - r_{DZ})$. The actual value of $2(r_{MZ} - r_{DZ})$ calculated from observations, in situations where the contribution of the dominance variance is not known, is therefore an upper limit estimate of the heritability. This estimate is twice the difference between two independent correlations, and consequently its standard error is relatively large (Smith, 1974). This kind of analysis has been applied to a variety of malformations and diseases, including clubfoot (Smith, 1970), schizophrenia (Gottesman & Shields, 1973) and psoriasis (Brandrup et al, 1978).

It is also possible to derive an upper limit estimate of heritability from MZ and DZ concordance rates without knowing the general population incidence of the trait since, when all the genetic variance is additive, $h^2 = (x_g - x_{MZ})/a = 2(x_g - x_{DZ})/a$, so that $x_g = 2x_{DZ} - x_{MZ}$. Using this value for x_g, reference to tables gives the corresponding value for a, and values for r can be calculated as already described.

This approach to the analysis of threshold characters assumes that the distribution of relatives on the underlying continuous scale of liability has the same variance as that of the general population. However, affected individuals (those falling above the threshold) form a truncated group with a skewed distribution, so that the distribution of relatives is likely to have both a lower variance than the general population and a skewed distribution also. These sources of bias are particularly likely to affect estimates of heritability from concordance in MZ twins. Elimination of this bias has shown that the proband concordance rate for MZ twins, which depends both on heritability and population incidence of the trait, will not be high unless heritability is very high, a heritability of 80%, for example, giving a proband concordance rate for MZ pairs of only 37% with a population frequency of 1% (Smith, 1970, 1972). A low concordance rate for MZ twins cannot therefore be taken as evidence that genetic factors are unimportant in the aetiology of a disease.

Complications in the interpretation of concordance rates can arise for a number of reasons, including a difference in incidence of the trait between the sexes, variable age of onset of a disease, or variation in diagnosis or ascertainment. These are discussed by Smith (1974).

Continuous variation

For continuous variables, resemblance between twins (either MZ or DZ) can be expressed by the intrapair variance, the variance within pairs, V_w. This is given by $V_w = \Sigma(X - Y)^2/2N$, with N degrees of freedom, where X and Y are values for members of each twin pair and N is the number of pairs. The interpair variance, the

variance between pairs, V_b, is then given as

$$V_b = \frac{1}{N-1}\left[\frac{\Sigma(X+Y)^2}{2} - \frac{(\Sigma(X+Y))^2}{2N}\right]$$

with $N-1$ degrees of freedom. The significance of a difference between MZ and DZ pairs in terms of either V_w or V_b can be tested by calculating the variance ratio, F, and referring to standard statistical tables, as has been done for plasma free and esterified cholesterol (Christian et al, 1976); but again, such a test is only able to indicate whether MZ twins are significantly more alike than DZ twins with respect to the character studied and does not provide a quantitative estimate of the genetic contribution to resemblance between members of twin pairs. A quantitative estimate can however be derived from these variances via the intraclass correlation, r_i, where $r_i = (V_b - V_w)/(V_b + V_w)$. The intraclass correlations for MZ and DZ pairs can then be used as described above to provide an upper limit estimate of the heritability. This kind of analysis has been applied, for example, to plasma creatine phosphokinase activity (Meltzer, 1978).

For characters unlikely to fulfil the criteria for parametric statistics, non-parametric methods can be applied to assess the relative similarity of MZ and DZ pairs. Such methods have recently been used to investigate the EEG reaction to ethanol (Propping, 1977) and the frequency of sister chromatid exchanges (Pedersen et al, 1979).

Holzinger's index

The use of Holzinger's index, H, to express the importance of hereditary factors in the aetiology of a disease, where $H = (V_{DZ} - V_{MZ})/V_{DZ} = (r_{MZ} - r_{DZ})/(1-r_{DZ})$, and where V_{MZ} and V_{DZ} are within pair variances for MZ and DZ pairs, is considered to be misleading and inappropriate for the analysis of twin data. Holzinger's index seriously underestimates the contribution of heredity and, for a given heritability calculated by conventional methods, increases with the population frequency of the disorder under study (Cavalli-Sforza & Bodmer, 1971; Smith, 1974). In spite of this, Holzinger's index has been used widely in the genetic literature.

The families of MZ twins

Monozygous twins have identical genotypes and consequently their children are related as half-sibs. The expected size and age range of the two sibships with MZ twin parents are the same, in contrast to conventional half-sibships, so that the families of MZ twins provide the opportunity for half-sib analysis under what for human investigations are remarkably controlled conditions (Nance & Corey, 1976; Haley et al, 1981). The particular advantage of half-sib analysis is that it allows different components of genetic and environmental variance to be estimated directly, without the need to resort to assumptions about whether or not particular components can be ignored. The method has been used recently to study the influence of X-linked genes on serum immunoglobulin M (Escobar et al,1979).

LIMITATIONS OF TWIN STUDIES

The previous section has shown briefly how resemblance between twins can be quantified and how, assuming that MZ and DZ pairs have equally similar environments, it is possible to eliminate the variance due to common environment in the analysis. However, very large numbers of twin pairs may be required to draw the simplest conclusions with confidence (Martin et al, 1978), and on the basis of twin data alone there is no way of knowing if the genetic component of resemblance has its basis in dominant or recessive genes, autosomal or X-linked genes, one or many loci. Furthermore, the assumption of equal similarity of environment for MZ and DZ pairs, although often the only course, is a source of error, since it is likely that general similarities of temperament and ability will result in a higher postnatal environmental correlation within MZ pairs than within DZ pairs.

On the other hand, although development in the same uterus at the same time clearly eliminates a major source of environmental variation, there is evidence that within pair prenatal environmental differences for dichorionic twins can be substantial. In terms of IQ and dermatoglyphic characteristics, greater within pair differences have been demonstrated for dichorionic as opposed to monochorionic MZ twins, whereas IQ differences for dichorionic MZ and DZ pairs were similar (Melnick et al, 1978; Reed et al, 1978). Intrauterine competition, particularly in MZ pairs, can also lead to marked differences in birth weight that can persist at least during early childhood (Wilson, 1979), and these differences could conceivably promote environmental differences from a variety of sources.

An additional source of confusion is that although the chromosomal complements of MZ twins are identical, the splitting of a single zygote into two could result in unequal distribution of cytoplasmic constituents. Therefore, whereas each DZ twin starts life with a full complement of cytoplasm, members of a monozygous pair may differ with respect to cytoplasmic factors. Such differences would contribute to what appear to be non-genetic differences between members of MZ pairs.

A potentially powerful way of studying the contribution of postnatal environment to resemblance between MZ twins involves the comparison of twins reared together with those reared apart. This comparison has been expressed by the ratio E, comparable to Holzin-

ger's H index, the ratio being given as $E = (V_a - V_t)/V_a$, where V_a and V_t are the within pair variances for twins reared apart and reared together respectively. The results of three such studies (Newman et al, 1937; Shields, 1962; Burt, 1966) have been summarised by Cavalli-Sforza & Bodmer (1971). There are two major difficulties with this approach. First, the number of MZ pairs reared apart is severely limited; and second, a considerable degree of similarity of environment is likely to persist even for separated pairs. The environmental differences found between members of separated pairs are therefore not comparable to the range of environmental variation found in the population as a whole. These problems make the interpretation of such studies difficult in general terms, particular phenotypic differences between members of separated MZ pairs often being attributable to known environmental differences specific to the pair involved. A further assumption is that non-genetic intrapair differences for MZ twins occur independently of genotype. However, the same environmental difference could produce varying phenotypic differences depending on the genotype of the pair involved.

Despite these limitations, or perhaps because of them, the major source of confusion in twin studies has been the notable lack of comparability of data collection, analysis and interpretation (Smith, 1974). This confusion should not obscure the fact that the twin method is a valuable approach to the study of genetic determination of a character, and, used in conjunction with information from other relatives, can be very informative.

REFERENCES

Brackenridge C J 1977 The secular variation of Australian twin births over fifty years. Annals of Human Biology 4: 559–64

Brandrup F, Hauge M, Henningsen K, Eriksen B 1978 Psoriasis in an unselected series of twins. Archives of Dermatology 114: 874–8

British Medical Journal 1976 Worldwide decline in dizygotic twinning. Vol. 1: 1553

*Bulmer M G 1970 The biology of twinning in man. Clarendon Press, Oxford

Bulmer M G 1976 Is Weinberg's method valid? Acta Geneticae Medicae et Gemellologiae 25: 25–8

Burt C 1966 The genetic determination of differences in intelligence: a study of monozygotic twins reared together and apart. British Journal of Psychology 57: 137–53

Carter-Saltzman L, Scarr S 1977 MZ or DZ? Only your blood grouping laboratory knows for sure. Behavior Genetics 7: 273–80

*Cavalli-Sforza L L, Bodmer W F 1971 The genetics of human populations. Freeman, San Francisco

Christian J C, Cheung S W, Kang K, Harmath F P, Huntzinger D J, Powell R C 1976 Variance of plasma free and esterified cholesterol in adult twins. American Journal of Human Genetics 28: 174–8

Cohen D J, Dibble E, Grawe J M, Pollin W 1975 Reliably separating identical from fraternal twins. Archives of General Psychiatry 32: 1371–5

Elwood J M 1978 Maternal and environmental factors affecting twin births in Canadian cities. British Journal of Obstetrics and Gynaecology 85: 351–8

*Emery A E H 1976 Methodology in medical genetics. Churchill Livingstone, Edinburgh

Escobar V, Corey L A, Bixler D, Nance W E, Biegel A 1979 The human X-chromosome and the levels of serum immunoglobulin M. Clinical Genetics 15: 221–7

Falconer D S 1960 Introduction to quantitative genetics. Oliver and Boyd, Edinburgh

Falconer D S 1965 The inheritance of liability to certain diseases estimated from the incidence among relatives. Annals of Human Genetics 29: 51–76

Gaines R E, Elston R C 1969 On the probability that a twin pair is monozygotic. American Journal of Human Genetics 21: 457–65

Gottesman I I, Shields J 1973 Genetic theorising and schizophrenia. British Journal of Psychiatry 122: 15–30

Haley C S, Jinks J L, Last K 1981 The monozygotic twin half-sib method for analysing maternal effects and sex-linkage in humans. Heredity 46: 227–38

James W H 1976 The possibility of a flaw underlying Weinberg's differential rule. Annals of Human Genetics 40: 197–9

James W H 1979 Is Weinberg's differential rule valid? Acta Geneticae Medicae et Gemellologiae 28: 69–71

Kasriel J, Eaves L 1976 The zygosity of twins. Further evidence on the agreement between diagnosis by blood groups and written questionnaires. Journal of Biosocial Science 8: 263–6

Lubs M-L E 1972 Empiric risks for genetic counseling in families with allergy. Journal of Pediatrics 80: 26–31

Martin N G, Eaves L J, Kearsey M J, Davies P 1978 The power of the classical twin study. Heredity 40: 97–116

Melnick M, Myrianthopoulos N C, Christian J C 1978 The effects of chorion type on variation in IQ in the NCPP twin population. American Journal of Human Genetics 30: 425–33

Meltzer H Y, Dorus E, Grunhaus L, Davis J M, Belmaker R 1978 Genetic control of human plasma creatine phosphokinase activity. Clinical Genetics 13: 321–6

Morton N E, Chung C S, Mi M P 1967 Genetics of interracial crosses in Hawaii. S. Karger, New York

Nance W E, Corey L A 1976 Genetic models for the analysis of data from the families of identical twins. Genetics 83: 811–26

Newman H H, Freeman F N, Holzinger K J 1937 Twins: a study of heredity and environment. University of Chicago Press, Chicago

Nielsen J, Homma A, Isaksen B, Bertelsen A 1978 Incidence of twin births in Denmark from 1911 to 1974 Acta Geneticae Medicae et Gemellologiae 27: 45–9

Pedersen C, Oláh E, Merrild U 1979 Sister chromatid exchanges in cultured peripheral lymphocytes from twins. Human Genetics 52: 281–94

Propping P 1977 Genetic control of ethanol action on the central nervous system. An EEG study in twins. Human Genetics 35: 309–34

Reed T, Uchida I A, Norton J A Jr, Christian J C 1978 Comparisons of dermatoglyphic patterns in monochorionic and dichorionic monozygotic twins. American Journal of Human Genetics 30: 383–91

Shields J 1962 Monozygotic twins brought up apart and brought up together. Oxford University Press, London

Smith C 1970 Heritability of liability and concordance in monozygous twins. Annals of Human Genetics 34: 85–91

Smith C 1972 Correlation in liability among relatives and concordance in twins. Human Heredity 22: 97–101

Smith C 1974 Concordance in twins: methods and interpretation. American Journal of Human Genetics 26: 454–66

Smith S M, Penrose L S 1955 Monozygotic and dizygotic twin diagnosis. Annals of Human Genetics 19: 273–89

Smith S M, Penrose L S, Smith C A B 1961 Mathematical tables for research workers in human genetics. J & A Churchill, London and Edinburgh

White C, Wyshak G 1964 Inheritance in human dizygotic twinning. New England Journal of Medicine 271: 1003–5

Wilson R S 1979 Twin growth: initial deficit, recovery, and trends in concordance from birth to nine years. Annals of Human Biology 6: 205–20

*General reference